chemistry proceedings

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Conference Report

14th Edition of the Nacional Organic Chemistry Meeting and 7th Edition of the Nacional Therapeutic Chemistry Meeting †

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† Presented at the 14th National Organic Chemistry Meeting and the 7th National Medicinal Chemistry Meeting, Caparica, Portugal, 20–22 April 2022.

Abstract: Once more under the auspices of the Sociedade Portuguesa de Química, two important fields of Chemistry are brought together into a single event, the 14th National Organic Chemistry Meeting and the 7th National Medicinal Chemistry Meeting. These conferences brought together both long-recognized experts and newcomers.

Keywords: organic synthesis; drug design; natural compounds; drug discovery; bioactive molecules; structure–activity relationship; Medicinal Chemistry; anticancer agents; photosensitizers

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1. Aim and Scope of the Meeting

The Scientific Committee brought together a wide range of specialists in the areas of Organic and Medicinal Chemistry, which allowed the high quality of the meeting that was evident in the scientific excellence of the works presented. The contributions include plenary lectures, invited oral communications, oral communications, keynotes, flash, and poster communications, where the main topics focused on organic synthesis, drug design, natural compounds, drug discovery, drug metabolism, and Medicinal Chemistry.

This approach between scientists is of great importance for the exchange of experiences and recent knowledge as well as different perspectives in the various areas of study, and it enhances collaboration between teams. This environment of scientific sharing took place in the relaxed atmosphere by the sea at Costa da Caparica.

2. Plenary Presentations

2.1. Incursions into Anticancer Drug Design and Drug Toxicity Elucidation: Strategies and Challenges

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Two major research avenues in our group are the design, synthesis and evaluation of new anticancer drugs and the elucidation of mechanisms of toxicity elicited by xenobiotic agents of therapeutic or environmental relevance. Selected recent examples from both approaches will be presented and discussed.

Emphasis will be placed on the combined use of in silico tools, chemical synthesis and proof-of-concept biochemical and biological testing to tackle epigenetic pathways

with relevance to cancer initiation and progression, to target glycolysis enzymes overexpressed in cancer cells, and to explore the potential of small organic molecules in cancer immunotherapy.

The use of mass spectrometry-based omics approaches to elucidate systemic effects of drugs on major biochemical pathways will be addressed in the context of proposed drug repurposing.

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2.2. How PROTAC Degraders Work: Molecular Recognition and Design Principles

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Proteolysis-targeting chimeras (PROTACs) are a new class of chemical tools and drugs that target disease-causing proteins for degradation. They are designed to harness the cell's natural disposal system (the ubiquitin-proteasome) to specifically remove proteins. A PROTAC is a two-headed (i.e., bifunctional) molecule where one end binds an enzyme (an E3 ubiquitin ligase) and the other binds the target protein, bringing the two proteins into close proximity as a ternary complex. The ligase is then able to label the target protein for ubiquitination and thus degradation by the cell's disposal system. Whereas conventional drugs only inhibit disease proteins by binding and locking up their most important functional parts for the duration of the drug's action, PROTACs can bind at any positions and rapidly cause the disease protein's permanent and long-lasting destruction. Due to this revolutionary mode of action, PROTACs can attack targets previously thought 'undruggable'. In this lecture, I will outline some key discoveries from my laboratory that have advanced the chemistry and structural biology of PROTACs, and these are providing fundamental insights into our understanding of their molecular recognition, mechanism of action and drug design.

2.3. Synthesis and Reactivity of Small-Ring Bicyclic Hydrocarbons

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Small ring bridged bicyclic hydrocarbons have become an important field of study in Medicinal Chemistry due to the beneficial physicochemical properties imparted by these rigid, Fsp3-rich motifs. Of particular interest is the bicyclo[1.1.1]pentane (BCP) framework, which has received significant attention as a surrogate/bioisostere for para-substituted benzene rings.

This lecture will discuss some recent advances from our laboratory on the synthesis of functionalized bicyclo[1.1.1]pentanes (BCPs) from [1.1.1]propellane, including the synthesis of BCPs featuring adjacent stereogenic centers [1,2], and a variety of heteroatom substituents (Figure 1) [3]. It will also include recent developments in the chemistry of the related strained hydrocarbon scaffold bicyclo[1.1.0]butane [4,5], which can serve as a precursor to a variety of other polysubstituted carbocycles, including BCPs.

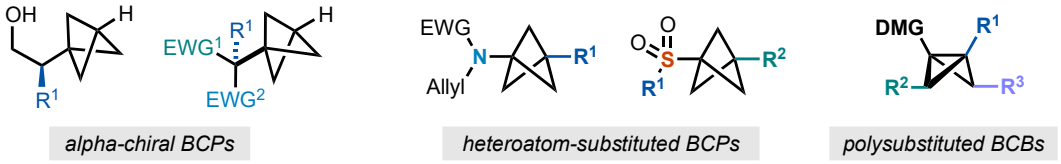

Figure 1. Bicyclic hydrocarbons of interest in Medicinal Chemistry research.

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References

1. Wong, M.L.J.; Sterling, A.J.; Mousseau, J.J.; Duarte, F.; Anderson, E.A. Direct catalytic asymmetric synthesis of α-chiral bicyclo[1.1.1]pentanes. *Nat. Commun.* **2021**, *12*, 1644.
2. Nugent, J.; Sterling, A.J.; Frank, N.; Mousseau, J.J.; Anderson, E.A. Synthesis of α-Quaternary Bicyclo[1.1.1]pentanes through Synergistic Organophotoredox and Hydrogen Atom Transfer Catalysis. *Org. Lett.* **2021**, *23*, 8628–8633.
3. Pickford, H.D.; Nugent, J.; Owen, B.; Mousseau, J.J.; Smith, R.C.; Anderson, E.A. Twofold Radical-Based Synthesis of N,C-Difunctionalized Bicyclo[1.1.1]pentanes. *J. Am. Chem. Soc.* **2021**, *143*, 9729–9736.
4. McNamee, R.E.; Haugland, M.M.; Nugent, J.; Chan, R.; Christensen, K.; Anderson, E.A. Synthesis of 1,3-disubstituted bicyclo[1.1.0]butanes via directed bridgehead functionalization. *Chem. Sci.* **2021**, *12*, 7480–7485.
5. McNamee, R.E.; Thompson, A.L.; Anderson, E.A. Synthesis and Applications of Polysubstituted Bicyclo[1.1.0]butanes. *J. Am. Chem. Soc.* **2021**, *143*, 21246–21251.

2.4. Harnessing Artificial Intelligence for De Novo Molecule Design

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Artificial intelligence (AI) is fueling computer-aided drug discovery [1,2]. Chemical language models [3,4] (CLMs) are one of the most recent additions to the medicinal chemist's toolkit for AI-driven molecule design. CLMs can be used to generate novel molecules in the form of strings (e.g., SMILES [5] or amino-acid sequences) without relying on human-engineered assembly rules. Thanks to such a 'rule-free' character, CLMs allow navigating the chemical space and generating focused chemical libraries. In multiple instances, CLMs have shown able to learn "grammar" rules for molecule construction and to implicitly capture "semantic" features, such as physicochemical properties, bioactivity, and chemical synthesizability [3,4,6–10]. This talk will illustrate some successful applications of CLMs to design novel bioactive compounds from scratch [6–10], e.g., natural-product-inspired modulators of nuclear receptors [9], and in combination with automated synthesis [10]. Moreover, the talk will provide a personal perspective on current limitations and future opportunities for AI in medicinal and organic chemistry to accelerate molecule discovery and chemical space exploration.

Acknowledgments: I would like to thank all co-authors whose contribution was key in the presented research [4], i.e., my former colleagues at the ETH Zurich (G. Schneider, M. Moret, L. Friedrich, B.J. Huisman, A.L. Button, C.S. Neuhaus, G. Gabernet, A.T. Müller, J.A. Hiss, K. Atz) and collaborators at the Goethe University Frankfurt and LMU (D. Merk and M. Helmstädter).

References

1. Jiménez-Luna, J.; Grisoni, F.; Weskamp, N.; Schneider, G. Artificial intelligence in drug discovery: recent advances and future perspectives. *Expert Opin. Drug Discov.* **2021**, *16*, 949–959, https://doi.org/10.1080/17460441.2021.1909567.
2. Chen, H.; Engkvist, O.; Wang, Y.; Olivecrona, M.; Blaschke, T. The rise of deep learning in drug discovery. *Drug Discov. Today* **2018**, *23*, 1241–1250, https://doi.org/10.1016/j.drudis.2018.01.039.
3. Yuan, W.; Jiang, D.; Nambiar, D.K.; Liew, L.P.; Hay, M.P.; Bloomstein, J.; Lu, P.; Turner, B.; Le, Q.; Tibshirani, R.; Khatri, P.; Moloney, M.G.; Koong, A.C. Chemical Space Mimicry for Drug Discovery. *J. Chem. Inf. Model.* **2017**, *57*, 875–882.
4. Segler, M.H.S.; Kogej, T.; Tyrchan, C.; Waller, M.P. Generating Focused Molecule Libraries for Drug Discovery with Recurrent Neural Networks. *ACS Central Sci.* **2017**, *4*, 120–131, https://doi.org/10.1021/acscentsci.7b00512.
5. Weininger, D. SMILES, a chemical language, and information system. 1. Introduction to methodology and encoding rules. *J. Chem. Inf. Model.* **1988**, *28*, 31–36.
6. Merk, D.; Friedrich, L.; Grisoni, F.; Schneider, G. *De Novo* Design of Bioactive Small Molecules by Artificial Intelligence. *Mol. Informatics* **2018**, *37*, https://doi.org/10.1002/minf.201700153.
7. Moret, M.; Friedrich, L.; Grisoni, F.; Merk, D.; Schneider, G. Generative molecular design in low data regimes. *Nat. Mach. Intell.* **2020**, *2*, 171–180, https://doi.org/10.1038/s42256-020-0160-y.
8. Grisoni, F.; Neuhaus, C.S.; Gabernet, G.; Müller, A.T.; Hiss, J.A.; Schneider, G. Designing Anticancer Peptides by Constructive Machine Learning. *ChemMedChem* **2018**, *13*, 1300–1302.
9. Moret, M.; Helmstädter, M.; Grisoni, F.; Schneider, G.; Merk, D. Beam Search for Automated Design and Scoring of Novel ROR Ligands with Machine Intelligence. *Angew. Chem. Int. Ed.* **2021**, *60*, 19477–19482.
10. Grisoni, F.; Huisman, B.J.; Button, A.L.; Moret, M.; Atz, K.; Merk, D.; Schneider, G. Combining generative artificial intelligence and on-chip synthesis for de novo drug design. *Sci. Adv.* **2021**, *7*, 3338.

2.5. Synthetic Transformations under Flow Conditions from Biomass Derived Synthetic Building Blocks

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The development of biorenewable chemical building blocks for chemical-based commodities is an important issue for a more sustainable synthetic organic chemistry [1,2]. In addition, performing reactions under continuous processes, using either high-scale or microflow devices, provides valuable benefits in terms of productivity, purity and safety derived from efficient reagent mixing, heat transfer and pressure control when compared to batch processes [3]. This laboratory has been involved in the development of some synthetic methodologies based on functional groups transformations under batch conditions. In this line, we will present some advances on the application of flow chemistry to some studied transformations of biomass-derived platforms under batch conditions such as oleuropein methanolysis [4], chemoselective modification of 5 hydroxymethylfurfural (HMF) derivatives (**1**) [5–7], heterogeneous catalyzed transformation of furfural to trans-4,5-diaminocyclopent-2-enones (**2**) [8–10], sequential photochemical rearrangement and hydration of N-alkyl pyridinium salts to bicyclic aziridines (**3**) [11,12] and enzymatic transformations (Figure 1).

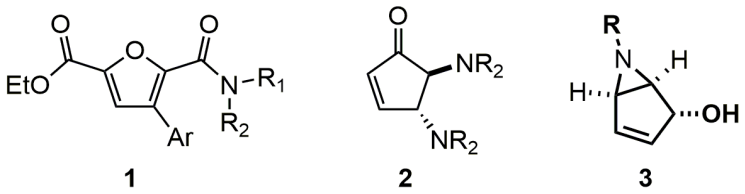

Figure 1. Examples of target synthetic building blocks.

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References

1. Gallezot, P. Conversion of biomass to selected chemical products. *Chem. Soc. Rev.* **2012**, *41*, 1538–1558, https://doi.org/10.1039/c1cs15147a.
2. Corma, A.; Iborra, S.; Velty, A. Chemical Routes for the Transformation of Biomass into Chemicals. *Chem. Rev.* **2007**, *107*, 2411–2502, https://doi.org/10.1021/cr050989d.
3. Plutschack, M.B.; Pieber, B.; Gilmore, K.; Seeberger, P.H. The Hitchhiker's Guide to Flow Chemistry. *Chem. Rev.* **2017**, *117*, 11796–11893.
4. Cavaca, L.; Rodrigues, C.; Simeonov, S.P.; Gomes, R.F.A.; Coelho, J.A.S.; Romanelli, G.P.; Sathicq, A.G.; Martínez, J.J.; Afonso, C.A.M. Valorization of Oleuropein via Tunable Acid-Promoted Methanolysis. *ChemSusChem* **2018**, *11*, 2300–2305, https://doi.org/10.1002/cssc.201800980.
5. Ravasco, J.; Monteiro, C.M.; Siopa, F.; Trindade, A.F.; Oble, J.; Poli, G.; Simeonov, S.P.; Afonso, C.A.M. Creating Diversity from Biomass: A Tandem Bio/Metal-Catalysis towards Chemoselective Synthesis of Densely Substituted Furans. *ChemSusChem* **2019**, *12*, 4629–4635, https://doi.org/10.1002/cssc.201902051.
6. Gomes, R.; Coelho, J.A.S.; Afonso, C.A.M. Direct Conversion of Activated 5-Hydroxymethylfurfural into δ-Lactone-Fused Cyclopentenones. *ChemSusChem* **2018**, *12*, 420–425, https://doi.org/10.1002/cssc.201802537.
7. Pardo, O.H.; Simeonov, S.P.; Peixoto, A.F.; Popova, M.D.; Lazarova, H.I.; Romanelli, G.P.; Martínez, J.J.; Freire, C.; Afonso, C.A.M. Efficient Continuous Production of the Biofuel Additive 5-(t-Butoxymethyl) Furfural from 5-Hydroxymethylfurfural. *Energy Technol.* **2019**, *7*, 1900780.
8. Gomes, R.F.A.; Esteves, N.R.; Coelho, J.A.S.; Afonso, C.A.M. Copper(II) Triflate As a Reusable Catalyst for the Synthesis of trans-4,5-Diamino-cyclopent-2-enones in Water. *J. Org. Chem.* **2018**, *83*, 7509–7513.
9. Pereira, J.G.; António, J.P.M.; Mendonça, R.; Gomes, R.F.A.; Afonso, C.A.M. Rediscovering aminal chemistry: copper(II) catalysed formation under mild conditions. *Green Chem.* **2020**, *22*, 7484–7490.
10. Gomes, R.F.A.; Cavaca, L.A.S.; Gonçalves, J.M.; Ramos, R.; Peixoto, A.F.; Arias-Serrano, B.I.; Afonso, C.A.M. Silica-Supported Copper for the Preparation of trans-4,5-Diamino-Cyclopent-2-Enones under Continuous Flow Conditions. *ACS Sust. Chem. Eng.* **2021**, *9*, 16038–16043.
11. Siopa, F.; António, J.P.M.; Afonso, C.A.M. Flow-Assisted Synthesis of Bicyclic Aziridines *via* Photochemical Transformation of Pyridinium Salts. *Org. Process Res. Dev.* **2018**, *22*, 551–556, https://doi.org/10.1021/acs.oprd.8b00036.
12. Fortunato, M.A.G.; Ly, C.-P.; Siopa, F.; Afonso, C.A.M. Process Intensification for the Synthesis of 6-Allyl-6-azabicyclo[3.1.0]hex-3-en-2-ol from 1-Allylpyridinium Salt Using a Continuous UV-Light Photoflow Approach. *Methods Protoc.* **2019**, *2*, 67, https://doi.org/10.3390/mps2030067.

2.6. Necessity Is the Mother of Invention: Natural Products and the Chemistry They Inspire

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The chemical synthesis of natural products provides an exciting platform from which to conduct fundamental research in chemistry and biology. Our group is currently pursuing the synthesis of several structurally complex natural products with a particular focus on the development of new convergent fragment coupling strategies. The densely packed arrays of heteroatoms and stereogenic centers that constitute these polycyclic targets challenge the limits of current technology and inspire the development of new synthetic strategies and tactics. This seminar will describe the latest progress in our target-directed synthesis endeavors [1].

Reference

1. Dibrell, S.E.; Tao, Y.; Reisman, S.E. Synthesis of Complex Diterpenes: Strategies Guided by Oxidation Pattern Analysis. *Acc. Chem. Res.* **2021**, *54*, 1360–1373.

3. Keynote Presentations

3.1. Accessing New Bioactive Molecules Using Sustainable Transition Metal Catalysis

Anthony J. Burke [1,2,3,4]

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Sustainable transition metal-catalyzed reactions have been used during the last 20 years as a convenient, versatile and robust methodology for accessing various types of structures, structures that are generally highly desirable for Medicinal Chemistry and pharmaceutical applications [1]. Over the last 10 years, we have developed novel transition metal-catalyzed processes that have provided a plethora of different key targets for pharmaceutical development (Figure 1). In this talk, the strategies that we used and the results we obtained will be discussed [2–4].

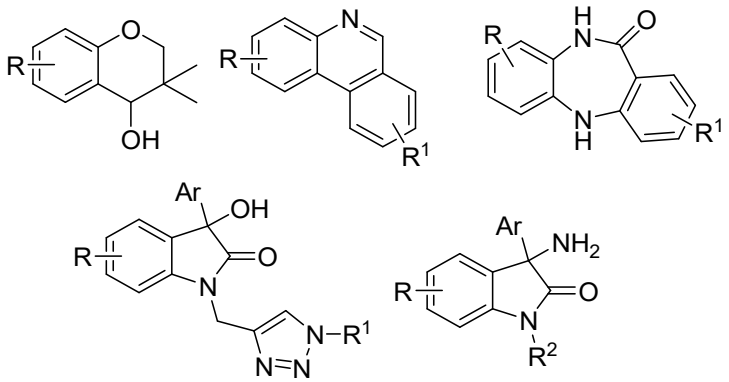

Figure 1. Examples of some targets developed.

References

1. Burke, A.J.; Marques, C.S.; Hermann, G.J.; Turner, N. (Eds.) *Active Pharmaceutical Ingredients in Synthesis, Catalytic Processes in Research and Development*; Wiley-VCH: Weinheim, Germany, 2018; ISBN 978-3-527-34241-9.
2. Marques, C.S.; López, O.; Bagetta, D.; Carreiro, E.P.; Petralla, S.; Bartolini, M.; Hoffmann, M.; Alcaroc, S.; Monti, B.; Bolognesi, M.L.; Decker, M.; Fernández-Bolaños, J.G.; Burke, A.J. N-1,2,3-triazole-isatin derivatives for cholinesterase and β-amyloid aggregation inhibition: A comprehensive bioassay study. *Bioorganic Chem.* **2020**, *98*, 103753.
3. Viana, H.; Marques, C.; Correia, C.A.; Gilmore, K.; Galvão, L.; Vieira, L.; Seeberger, P.H.; Burke, A.J. Novel Palladium-Catalyzed Intramolecular Addition of Aryl Bromides to Aldehydes as Key to the Synthesis of 3,3-Dimethylchroman-4-ones and 3,3-Dimethylchroman-4-ols. *ChemistrySelect* **2018**, *3*, 11333–11338, https://doi.org/10.1002/slct.201802889.
4. Marques, C.S.; Burke, A.J. Modular Catalytic Synthesis of 3-Amino-3-aryl-2-oxindoles: Rh Catalysis with Isatin-Derived N-Boc-Protected Ketimines. *Eur. J. Org. Chem.* **2016**, *2016*, 806–812.

3.2. Photodynamic Activity of Porphyrin Derivatives: Synthesis and Applications

M. Amparo F. Faustino [1,*], Cristina J. Dias [1], Mariana Q. Mesquita [1], Ana S. Joaquinito [1], Letícia D. Costa [1], Sara Gamelas [1], Carla Santos [1], Kelly A. D. F. Castro [1], Maria Bartolomeu [2], Cátia Vieira [2], Lúcia Maciel [2], Ana T. P. Gomes [2], Leandro M. O. Lourenço [1], Catarina I. V. Ramos [1], Nuno M. M. Moura [1] and Carlos J. P. Monteiro [1]

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Tetrapyrrolic macrocycles (e.g., porphyrins, chlorins, etc.) are a ubiquitous class of compounds in nature with unique physicochemical properties and an established role in crucial biological functions such as respiration, electron transfer and photosynthesis [1]. The adequate functionalization of these natural or synthetic macrocycles at different positions (meso and/or beta-pyrrolic positions) or their immobilization on different supports are responsible for their successful applications in several fields [2]. In particular, some of these derivatives have shown remarkable properties and significant potential to be used as photosensitizers in photodynamic therapy against tumoral cells, microbial cells in planktonic and biofilms forms, viruses and parasites [3–5]. This success takes advantage among others of their high molar extinction coefficients in the visible region and good quantum yields of singlet oxygen production (1O2). In this communication, an overview of some of the recent synthetic advances obtained in our group to obtain immobilized and non-immobilized meso-arylporphyrins derivatives and applications related with their photodynamic efficiency under different contexts will be addressed and discussed [6–15].

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References

1. Almeida, A.; Cunha, A.; Faustino, M.A.F.; Tomé, A.C.; Neves, M.G.P.M.S. Chapter 5. Porphyrins as Antimicrobial Photosensitizing Agents. *Royal Soc. Chem.* **2011**, 83–160, https://doi.org/10.1039/9781849733083-00083.
2. Kadish, K.M., Smith K.M., Guilard, R. (Eds.) *Handbook of Porphyrin Science, With Applications to Chemistry, Physics, Materials Science, Engineering, Biology and Medicine*; World Scientific Publishing Company: Singapore, 2016, Volume 32.
3. Mesquita, M.Q.; Dias, C.J.; Neves, M.G.P.M.S.; Almeida, A.; Faustino, M.A.F. Revisiting Current Photoactive Materials for Antimicrobial Photodynamic Therapy. *Molecules* **2018**, *23*, 2424, https://doi.org/10.3390/molecules23102424.
4. Mesquita, M.; Dias, C.; Gamelas, S.; Fardilha, M.; Neves, M.G.; Faustino, M.A.F. An insight on the role of photosensitizer nanocarriers for Photodynamic Therapy. *Int. J. Mol. Sci.* **2018**, *90*, 1101–1130, https://doi.org/10.1590/0001-3765201720170800.
5. Mesquita, M.Q.; Dias, C.J.; Neves, M.G.P.M.S.; Almeida, A.; Faustino, M.A.F. The Role of Photoactive Materials Based on Tetrapyrrolic Macrocycles in Antimicrobial Photodynamic Therapy. *Porphyr. Sci.* **2022**, 201–277, https://doi.org/10.1142/9789811246760_0221.
6. Castro, K.A.D.F.; Costa, L.D.; Prandini, J.A.; Biazzotto, J.C.; Tomé, A.C.; Hamblin, M.R.; Neves, M.D.G.P.M.S.; Faustino, M.A.F.; Da Silva, R.S. The Photosensitizing Efficacy of Micelles Containing a Porphyrinic Photosensitizer and KI against Resistant Melanoma Cells. *Chem. A Eur. J.* **2020**, *27*, 1990–1994, https://doi.org/10.1002/chem.202004389.
7. Mesquita, M.Q.; Ferreira, A.R.; Neves, M.D.G.P.; Ribeiro, D.; Fardilha, M.; Faustino, M.A. Photodynamic therapy of prostate cancer using porphyrinic formulations. *J. Photochem. Photobiol. B Biol.* **2021**, *223*, 112301, https://doi.org/10.1016/j.jphotobiol.2021.112301.
8. Bartolomeu, M.; Oliveira, C.; Pereira, C.; Neves, M.; Faustino, M.; Almeida, A. Antimicrobial Photodynamic Approach in the Inactivation of Viruses in Wastewater: Influence of Alternative Adjuvants. *Antibiotics* **2021**, *10*, 767, https://doi.org/10.3390/antibiotics10070767.
9. Lopes, M.M.; Bartolomeu, M.; Gomes, A.T.P.C.; Figueira, E.; Pinto, R.; Reis, L.; Balcão, V.M.; Faustino, M.A.F.; Neves, M.G.P.M.S.; Almeida, A. Antimicrobial Photodynamic Therapy in the Control of *Pseudomonas syringae* pv. *actinidiae* Transmission by Kiwifruit Pollen. *Microorganisms* **2020**, *8*, 1022, https://doi.org/10.3390/microorganisms8071022.
10. Castro, K.A.D.F.; Brancini, G.T.P.; Costa, L.D.; Biazzotto, J.C.; Faustino, M.A.F.; Tomé, A.C.; Neves, M.G.P.M.S.; Almeida, A.; Hamblin, M.R.; da Silva, R.S.; et al. Efficient photodynamic inactivation of *Candida albicans* by porphyrin and potassium iodide co-encapsulation in micelles. *Photochem. Photobiol. Sci.* **2020**, *19*, 1063–1071, https://doi.org/10.1039/d0pp00085j.
11. Braz, M.; Salvador, D.; Gomes, A.T.; Mesquita, M.Q.; Faustino, M.A.F.; Neves, M.G.P.; Almeida, A. Photodynamic inactivation of methicillin-resistant Staphylococcus aureus on skin using a porphyrinic formulation. *Photodiagnosis Photodyn. Ther.* **2020**, *30*, 101754, https://doi.org/10.1016/j.pdpdt.2020.101754.
12. Dias, C.J.; Sardo, I.; Moura, N.M.; Felgueiras, J.; Neves, M.G.P.; Fardilha, M.; Faustino, M.A.F. An efficient synthetic access to new uracil-alditols bearing a porphyrin unit and biological assessment in prostate cancer cells. *Dye. Pigment.* **2019**, *173*, 107996, https://doi.org/10.1016/j.dyepig.2019.107996.
13. Castro, K.A.; Moura, N.M.; Simões, M.M.; Cavaleiro, J.A.; Faustino, M.D.A.F.; Cunha, Â.; Paz, F.A.A.; Mendes, R.F.; Almeida, A.; Freire, C.S.; et al. Synthesis and characterization of photoactive porphyrin and poly(2-hydroxyethyl methacrylate) based materials with bactericidal properties. *Appl. Mater. Today* **2019**, *16*, 332–341, https://doi.org/10.1016/j.apmt.2019.06.010.
14. Vieira, C.; Santos, A.; Mesquita, M.Q.; Gomes, A.T.P.C.; Neves, M.G.P.M.S.; Faustino, M.A.F.; Almeida, A. Advances in aPDT based on the combination of a porphyrinic for-

mulation with potassium iodide: Effectiveness on bacteria and fungi planktonic/biofilm forms and viruses. *J. Porphyrins Phthalocyanines* **2019**, *23*, 534–545, https://doi.org/10.1142/s1088424619500408.
15. Marciel, L.; Mesquita, M.Q.; Ferreira, R.; Moreira, B.; Neves, M.G.P.; Faustino, M.A.F.; Almeida, A. An efficient formulation based on cationic porphyrins to photoinactivate *Staphylococcus aureus* and *Escherichia coli*. *Futur. Med. Chem.* **2018**, *10*, 1821–1833, https://doi.org/10.4155/fmc-2018-0010.

3.3. Asymmetric Catalysis: From Laboratory to Scale-Up Development

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Over the past few years, significant research has been directed toward the development of new methods for synthetic efficiency and atom economical processes. Among them, the potential of transition metal-catalyzed reactions has been steadily demonstrated, as they provide a direct and selective way toward the synthesis of highly valuable products. We have been engaged in a project dedicated to the development of catalytic methods for the synthesis of bio-relevant targets. More specifically, we have been interested in asymmetric reductions such as hydrogenation [1,2] using SYNPHOS and DIFLUORPHOS [3–5] developed in our group as chiral ligands and transfer hydrogenation reactions [6], which provide important catalytic approaches to fine chemicals (Figure 1). In this context, our contribution to this field is the development of novel organometallic complexes to access biorelevant targets. Some recent applications in this field will be presented [7–10].

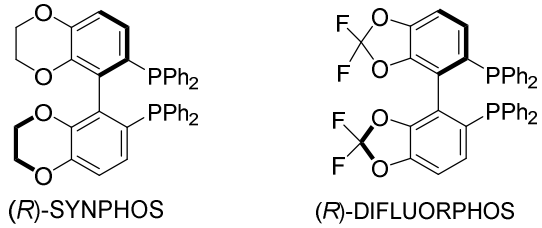

Figure 1. SYNPHOS and DIFLUORPHOS developed in the group.

References

1. Vidal, V.; Phansavath, P. *Asymmetric Hydrogenation and Transfer Hydrogenation*; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2021; ISBN 978-3-527-34610-3.
2. Genêt, J.; Phansavath, P.; Ratovelomanana-Vidal, V. Asymmetric Hydrogenation: Design of Chiral Ligands and Transition Metal Complexes. Synthetic and Industrial Applications. *Isr. J. Chem.* **2021**, *61*, 409–426, https://doi.org/10.1002/ijch.202100023.
3. Genet, J.-P.; Ayad, T.; Ratovelomanana-Vidal, V. Electron-Deficient Diphosphines: The Impact of DIFLUORPHOS in Asymmetric Catalysis. *Chem. Rev.* **2014**, *114*, 2824–2880, https://doi.org/10.1021/cr4003243.
4. Jeulin, S.; De Paule, S.D.; Ratovelomanana-Vidal, V.; Genêt, J.-P.; Champion, N.; Dellis, P. Difluorphos, an Electron-Poor Diphosphane: A Good Match Between Electronic and Steric Features. *Angew. Chem. Int. Ed.* **2003**, *43*, 320–325, https://doi.org/10.1002/anie.200352453.
5. Jeulin, S.; de Paule, S.D.; Ratovelomanana-Vidal, V.; Genêt, J.-P.; Champion, N.; Dellis, P. Chiral biphenyl diphosphines for asymmetric catalysis: Stereoelectronic design and industrial perspectives. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5799–5804, https://doi.org/10.1073/pnas.0307620101.
6. Ayad, T.; Phansavath, P.; Ratovelomanana-Vidal, V. Transition-Metal-Catalyzed Asymmetric Hydrogenation and Transfer Hydrogenation: Sustainable Chemistry to Access Bioactive Molecules. *Chem. Rec.* **2016**, *16*, 2754–2771, https://doi.org/10.1002/tcr.201600100.

7. Molina-Betancourt, R.; Phansavath, P.; Ratovelomanana-Vidal, V. Rhodium-Catalyzed Asymmetric Transfer Hydrogenation/Dynamic Kinetic Resolution of 3-Benzylidene-Chromanones. *Org. Lett.* **2021**, *23*, 1621–1625.
8. Molina-Betancourt, R.; Phansavath, P.; Ratovelomanana-Vidal, V. Ru(II)-Catalyzed Asymmetric Transfer Hydrogenation of 3-Fluorochromanone Derivatives to Access Enantioenriched cis-3-Fluorochroman-4-ols through Dynamic Kinetic Resolution. *J. Org. Chem.* **2021**, *86*, 12054–12063.
9. He, B.; Phansavath, P.; Ratovelomanana-Vidal, V. Kinetic resolution of 2-aryl-2,3-dihydroquinolin-4(1H)-one derivatives by rhodium-catalysed asymmetric transfer hydrogenation. *Org. Chem. Front.* **2021**, *8*, 2504–2509.
10. Westermeyer, A.; Guillamot, G.; Phansavath, P.; Ratovelomanana-Vidal, V. Synthesis of Enantioenriched β-Hydroxy-γ-Acetal Enamides by Rhodium-Catalyzed Asymmetric Transfer Hydrogenation. *Org. Lett.* **2020**, *22*, 3911–3914.

3.4. New Chemistries for Stimuli-Responsive Targeting Drug Conjugates

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Targeting drug conjugates emerged as a powerful class of chemotherapeutic agents that are capable of sparing healthy tissues by liberating the cytotoxic payload upon specific antigen recognition (Figure 1). A considerable body of work in this field highlighted that targeting drug conjugates lead to therapeutic efficacy, and they correlate well with the conjugate homogeneity and activation of the drug at the diseased site. Therefore, the linker technology used to connect both functions contributes decisively to the therapeutic usefulness of these constructs. In this communication, we will present our most recent finding on the design of functional likers for targeting drug conjugates based on boron complexes (B-complexes) [1] that can be modulated to exhibit fluorescence and to respond to glutathione, pH or reactive oxygen species stimulus [2–4].

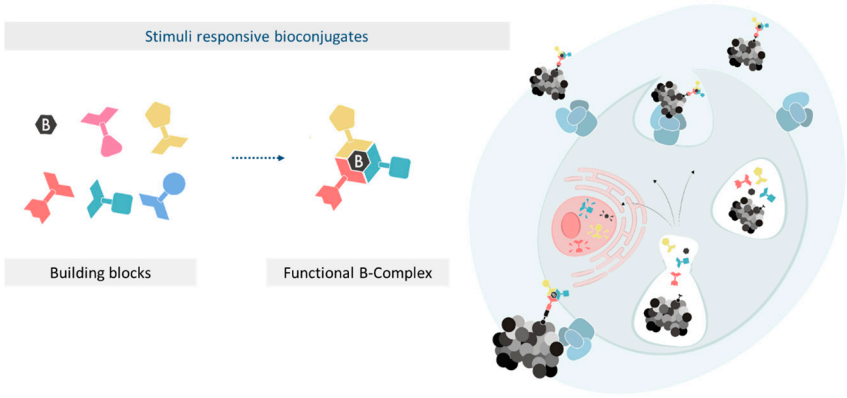

Figure 1. Modular, stimuli-responsive targeting drug conjugates.

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References

1. António, J.P.M.; Russo, R.; Carvalho, C.P.; Cal, P.M.S.D.; Gois, P.M.P. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates. *Chem. Soc. Rev.* **2019**, *48*, 3513–3536.

2. Lopes, R.M.R.M.; Ventura, A.E.; Silva, L.C.; Faustino, H.; Gois, P.M.P. *N,O*-Iminoboronates: Reversible Iminoboronates with Improved Stability for Cancer Cells Targeted Delivery. *Chem. A Eur. J.* **2018**, *24*, 12495–12499, https://doi.org/10.1002/chem.201802515.
3. Santos, F.M.F.; Matos, A.I.; Ventura, A.E.; Gonçalves, J.; Veiros, L.F.; Florindo, H.F.; Gois, P.M.P. Modular Assembly of Reversible Multivalent Cancer-Cell-Targeting Drug Conjugates. *Angew. Chem. Int. Ed.* **2017**, *56*, 9346–9350, https://doi.org/10.1002/anie.201703492.
4. António, J.P.M.; Carvalho, J.I.; André, A.S.; Dias, J.N.R.; Aguiar, S.I.; Faustino, H.; Lopes, R.M.R.M.; Veiros, L.F.; Bernardes, G.J.L.; da Silva, F.A.; Gois, P.M.P. Diazaborines Are a Versatile Platform to Develop ROS-Responsive Antibody Drug Conjugates. *Angew. Chemie Int. Ed. Engl.* **2021**, *60*, 25914–25921.

3.5. On the Green Road toward the Synthesis of Challenging N-Heterocycles

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Azaindoles are bioisosteres of the indole nucleus, a privileged structure, which have enticed the interest of the scientific community for their physicochemical and pharmacological properties. Azaindoles are rare in nature and highly interesting in Medicinal Chemistry and drug discovery programs. This is mainly due to the fact that their solubility, lipophilicity, target binding and ADME-tox properties can be modulated and tuned, constituting an enormous advantage over other heterocyclic compounds [1]. However, the synthesis of azaindoles is challenging. The electron-deficient nature of the pyridine ring alters the electronic properties of the conjugated system in such a way that many classic indole synthetic methods are not as efficient or simply do not work. Due to their important value, we have developed methods for the synthesis of azaindoles, relying on palladium-catalyzed cross-coupling reactions and developed different practical approaches compatible with all azaindole isomers [2,3]. Our group has been focused on metal-catalyzed cross-coupling reactions for the straightforward synthesis of azaindoles from commercially available aminopyridines. In particular, we have been exploring Pd-catalyzed one-pot methodologies such as the C-N cross-coupling/Heck reaction [4] also with Pd-nanocatalysts [5]; the *N*-arlyation/Sonogashira/cyclization reaction [6]; and Pd-catalyzed C-N cross-coupling/C-H functionalization [7] (Figure 1). Herein, we will present our latest achievements on the one-pot reactions and simple protocols toward not easy to make heterocycles.

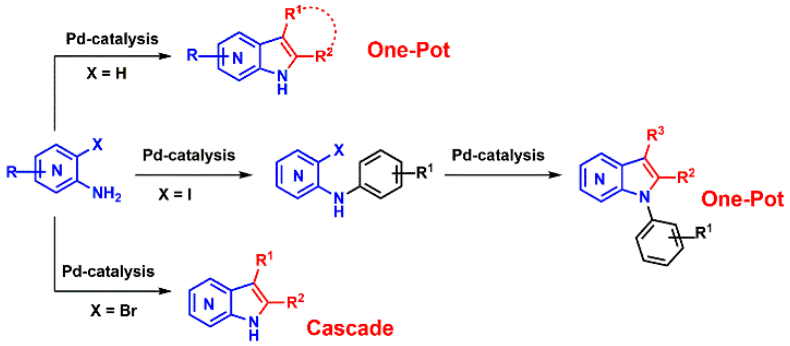

Figure 1. Novel metal-catalyzed syntheses of *N*-heterocycles.

Funding: Thanks to the Fundação para a Ciência e Tecnologia (FC&T) (project PTDC/QUI-QOR/**0712**/2020). This work was supported by the Associate Laboratory for Green Chemistry LAQV, which is financed by national funds from the FCT/MCTES (UID/QUI/50006/2020) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER—007265). The National NMR Facility is supported by the FCT (ROTEIRO/

0031/2013—PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI, and PORL and FCT through PIDDAC).

References

1. Mérour, J.-Y.; Buron, F.; Plé, K.; Bonnet, P.; Routier, S. The Azaindole Framework in the Design of Kinase Inhibitors. *Molecules* **2014**, *19*, 19935–19979, https://doi.org/10.3390/molecules191219935.
2. Pires, M.J.D.; Poeira, D.L.; Marques, M.M.B. Metal-Catalyzed Cross-Coupling Reactions of Aminopyridines. *Eur. J. Org. Chem.* **2015**, *2015*, 7197–7234, https://doi.org/10.1002/ejoc.201500952.
3. Santos, A.S.; Mortinho, A.C.; Marques, M.M.B. Metal-Catalyzed Cross-Coupling Reactions on Azaindole Synthesis and Functionalization. *Molecules* **2018**, *23*, 2673, https://doi.org/10.3390/molecules23102673.
4. Pires, M.J.D.; Poeira, D.L.; Purificação, S.I.; Marques, M.M.B. Synthesis of Substituted 4-, 5-, 6-, and 7-Azaindoles from Aminopyridines via a Cascade C–N Cross-Coupling/Heck Reaction. *Org. Lett.* **2016**, *18*, 3250−3253.
5. Rodriguez-Oliva, I.; Losada-Garcia, N.; Santos, A.S.; Marques, M.M.B.; Palomo, J.M. Palladium Nanocatalysts for Cascade C−N Cross-Coupling/Heck Reaction. *Asian J. Org. Chem.* **2021**, *10*, 872–878.
6. Purificação, S.I.; Pires, M.J.D.; Rippel, R.; Santos, A.S.; Marques,M.M.B. One-Pot Synthesis of 1,2-Disubstituted 4-, 5-, 6-, and 7-Azaindoles from Amino-o-halopyridines via N-Arylation/Sonogashira/Cyclization Reaction. *Org. Lett.* **2017**, *19*, 5118−5121.
7. Santos, A.S.; Martins, M.M.; Mortinho, A.C.; Silva, A.M.S.; Marques, M.M.B. Exploring the reactivity of halogen-free aminopyridines in one-pot palladium-catalyzed C–N cross-coupling/C–H functionalization. *Tetrahedron Lett.* **2020**, *61*, 152303–152308.

3.6. Discovery of Submicromolar Inhibitors of the Virulence Factor LasB from Pseudomonas Aeruginosa Using Rational Design

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Pseudomonas aeruginosa is a Gram-negative bacterium, typically affecting the lungs, urinary tract and wounds, leading to severe infections. Treatment is becoming increasingly challenging due to the rapid emergence of drug-resistant strains. Recently, significant efforts have been put into the development of the 'pathoblockers'—agents capable of blocking bacterial virulence by disarming the pathogen rather than killing it. Among a vast number of virulence factors secreted by *P. aeruginosa*, elastase (LasB) plays a crucial role in the infection process and is considered as a promising target for the development of new inhibitors [1,2].

Here, we report on the structure-based optimization of our *N*-arylmercaptoacetamides, resulting in two highly potent chemical classes (Figure 1). We pursued rigidification and a structure-based fragment growing. Freezing the active conformation in the form of succinimides enhanced the activity toward LasB two-fold compared to our previously published inhibitors and improved chemical stability with regard to disulfide formation [3,4]. On the other hand, exploiting structure-based design, fragment growing of the original hit led to a substantial 16-fold boost in activity [5]. In addition to the substantial increase in the potency, our new derivatives show no cytotoxicity and are highly selective for the bacterial metalloproteases over human matrix metalloproteases. Having demonstrated an excellent in vivo effect in a *Galleria mellonella* infection model, one of the selected inhibitors was further evaluated for its pharmacokinetic profile in mice and was subjected to an

advanced SafetyScreen44 panel. Taken together, our inhibitors hold a lot of potential as novel therapeutics in the form of an adjunctive therapy for *P. aeruginosa*-derived infections.

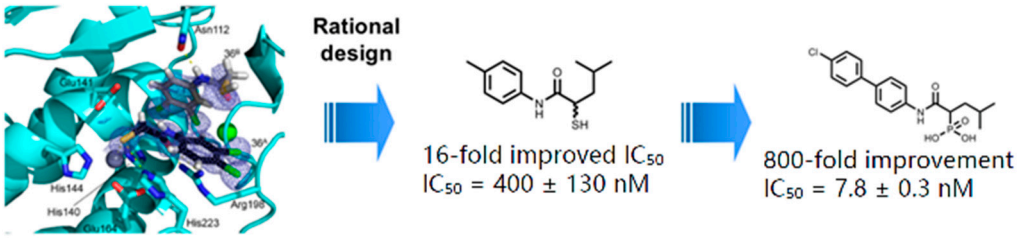

Figure 1. Structure-based optimization of LasB inhibitors.

References

1. Bassetti, M.; Vena, A.; Croxatto, A.; Righi, E.; Guery, B. How to manage Pseudomonas aeruginosa infections. *Drugs Context.* **2018**, *7*, 212527, https://doi.org/10.7573/dic.212527.
2. Calvert, M.B.; Jumde, V.R.; Titz, A. Pathoblockers or antivirulence drugs as a new option for the treatment of bacterial infections. *Beilstein J. Org. Chem.* **2018**, *14*, 2607–2617, https://doi.org/10.3762/bjoc.14.239.
3. Kany, A.M.; Sikandar, A.; Haupenthal, J.; Yahiaoui, S.; Maurer, C.K.; Proschak, E.; Köhnke, J.; Hartmann, R.W. Binding Mode Characterization and Early in Vivo Evaluation of Fragment-Like Thiols as Inhibitors of the Virulence Factor LasB from Pseudomonas aeruginosa. *ACS Infect. Dis.* **2018**, *4*, 988–997, https://doi.org/10.1021/acsinfecdis.8b00010.
4. Konstantinović, J.; Yahiaoui, S.; Alhayek, A.; Haupenthal, J.; Schönauer, E.; Andreas, A.; Kany, A.M.; Müller, R.; Koehnke, J.; Berger, F.K.; Bischoff, M.; Hartmann, R.W.; Brandstetter, H.; Hirsch, A.K.H. N-Aryl-3-mercaptosuccinimides as Antivirulence Agents Targeting Pseudomonas aeruginosa Elastase and Clostridium Collagenases. *J. Med. Chem.* **2020**, *63*, 8359–8368, https://doi.org/10.1021/acs.jmedchem.0c00584.
5. Ducho, C.; Hartmann, R.W.; Haupenthal, J.; Hirsch, A.K.H.; Kany, A.M.; Kaya, C.; Konstantinović, J.; Voos, K.; Walter, I.; Yahiaoui, S. EP 20 192 608.6. Patent Pending. Germany, 25 August 2020.

4. Invited Oral Presentations

4.1. New Purine-Based Compounds as Adenosine Receptor Antagonists: Synthesis and Structure–Activity Relationships

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Adenosine receptors (Ars), namely A_1, A_{2A}, A_{2B} and A_3, are widely recognized as potential therapeutic targets for drug development against different clinical disorders [1,2]. For example, A_1 ligands are under development for cardiovascular diseases, pain indications and glaucoma [3,4]. In addition, A_{2B} antagonists and dual A_{2B}/A_3 antagonists are being investigated for their use in asthma, diabetes, and cancer. A_{2A} agonists are in clinical trials for cardiac imaging diagnostic and wound healing, and some have already been approved for cardiac perfusion imaging [3,5–7], while A_{2A} antagonists are approved as

adjunctive for treating Parkinson's disease [8] or are under development for the treatment of cancer [9]. Finally, A_3 agonists have been linked to inflammatory diseases, such as rheumatoid arthritis and psoriasis, liver cancer, hepatitis, and liver regeneration, and they showed efficacy in clinical trials for dry eye syndrome [3,5–7,10]. Although numerous adenosine receptor inhibitors have been developed worldwide, achieving target selectivity is still a big hurdle in drug development that justifies the search for new potent and selective ligands.

Our research group recently identified a new purine-based scaffold with high affinity for AR. The SAR study showed that the potency and selectivity were dependent on the substituent groups present on C2, C6 and N9 of the purine nucleus [11].

In the present work, we will present the synthesis and the SAR analysis of a new set of derivatives.

Funding: We thank the Spanish Ministerio de Ciencia e Innovación (grants HF2007-0055 and BIO2008-02329) and the Fundação para a Ciência e Tecnologia through the Chemistry Research Centre of the University of Minho (ref. UID/QUI/00686/2018 and UID/QUI/00686/2020) and the PhD grant SFRH/BD/85937/2012.

References

1. Jacobson, K.; Gao, Z.-G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discov.* **2006**, *5*, 247–264, https://doi.org/10.1038/nrd1983.
2. Zhong, H.; Belardinelli, L.; Maa, T.; Feoktistov, I.; Biaggioni, I.; Zeng, D. A_{2B} Adenosine Receptors Increase Cytokine Release by Bronchial Smooth Muscle Cells. *Am. J. Respir. Cell Mol. Biol.* **2004**, *30*, 118–125, https://doi.org/10.1165/rcmb.2003-0118oc.
3. Fredholm, B.B.; IJzerman, A.P.; Jacobson, K.A.; Linden, J.; Müller, C.E. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and Classification of Adenosine Receptors—An Update. *Pharmacol. Rev.* **2011**, *63*, 1–34, https://doi.org/10.1124/pr.110.003285.
4. Jacobson, K.A.; Müller, C.E. Medicinal chemistry of adenosine, P2Y and P2X receptors. *Neuropharmacology* **2015**, *104*, 31–49, https://doi.org/10.1016/j.neuropharm.2015.12.001.
5. Müller, C.E.; Jacobson, K. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochim. Biophys. Acta (BBA) Biomembr.* **2011**, *1808*, 1290–1308, https://doi.org/10.1016/j.bbamem.2010.12.017.
6. Adenosine Receptors in Health and Disease. **2009**, https://doi.org/10.1007/978-3-540-89615-9.
7. Kellici, T.F.; Liapakis, G.; Tzakos, A.G.; Mavromoustakos, T. Pharmaceutical Compositions for Antihypertensive Treatments: A Patent Review. *Expert Opin. Ther. Pat.* **2015**, *25*, 1305–1317. https://doi.org/10.1517/13543776.2015.1086337
8. Saini, A.; Patel, R.; Gaba, S.; Singh, G.; Gupta, G.; Monga, V. Adenosine receptor antagonists: Recent advances and therapeutic perspective. *Eur. J. Med. Chem.* **2021**, *227*, 113907, https://doi.org/10.1016/j.ejmech.2021.113907.
9. Vijayan, D.; Young, A.; Teng, M.W.; Smyth, M.J. Targeting immunosuppressive adenosine in cancer. *Nat. Cancer* **2017**, *17*, 709–724, https://doi.org/10.1038/nrc.2017.86.
10. Sousa, J.B.; Fresco, P.; Diniz, C.; Goncalves, J. Adenosine Receptor Ligands on Cancer Therapy: A Review of Patent Literature. *Recent Patents Anti-Cancer Drug Discov.* **2018**, *13*, 40–69, https://doi.org/10.2174/1574892812666171108115959.
11. Areias, F.; Correia, C.; Rocha, A.; Brea, J.; Castro, M.; Loza, M.I.; Proença, M.F.; Carvalho, M.A. 2-Aryladenine derivatives as a potent scaffold for A1, A3 and dual A1/A3 adenosine receptor antagonists: Synthesis and structure-activity relationships. *Bioorganic Med. Chem.* **2019**, *27*, 3551–3558, https://doi.org/10.1016/j.bmc.2019.06.034.

4.2. Granzyme B-Specific Peptide Substrates as Fluorescent Reporters in a Drug Delivery System for Colorectal Cancer

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Among all cancer types, colorectal cancer (CRC) is the first non-gender specific cancer type by incidence in Portugal, constituting a serious societal burden especially in the northern region with the highest standardized death rates by CRC in the country [1]. Currently, there is a paradigm shift in cancer therapies from being focused on directly depleting tumoral cells through chemo and/or radio-therapy (which lack specificity and cause damage to healthy tissues) to activating the patient's immune system (cytotoxic T lymphocytes, CTLs, and natural killer, NK, cells) so that the patient can address the tumoral challenge himself [2].

Our recent research is focused on a theranostic probe to impact the prognosis of CRC through a combination of therapy and molecular imaging by the preparation, characterization and in vitro validation of an immunostimulant drug delivery system based on magnetic nanoparticles provided with a fluorescent reporting system so that the response to treatment can be monitored by magnetic resonance imaging and optical fluorescence imaging [3–5]. The fluorescent reporting system targets the presence of Granzyme B (GzmB), which is a serine protease and a potent inducer of apoptosis in target cells when released by CTLs and NK cells, representing one of the two dominant mechanisms by which these cells mediate cancer cell death [6].

In this communication, and since GzmB has a preference for cleaving after aspartic acid, we report the synthesis of specific peptide substrates for GzmB containing aspartic acid, which were labeled with an appropriate donor/acceptor FRET pair, namely with a near-infrared fluorophore at one terminal and with a fluorescence quencher at the other terminal. The synthesized fluorophore and peptides were fully characterized by the usual spectroscopic techniques.

The results of the in vitro assays for monitoring GzmB activity through fluorescence-based techniques will be presented, as upon the processing of the peptidic sequence by GzmB, the fluorophore and quencher are separated, and an optical signal is measured. This fluorescent signal can be correlated to the immune system activation and to a positive response of the patient to the treatment.

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References

1. RON. Registo Oncológico Nacional 2018; 2021. ISBN 978-989-95380-3-0. Available online: https://ron.min-saude.pt/pt/biblioteca/publicacoes-ron/ron-2018/ (accessed on 19 December 2021).
2. Darroudi, M.; Gholami, M.; Rezayi, M.; Khazaei, M. An overview and bibliometric analysis on the colorectal cancer therapy by magnetic functionalized nanoparticles for the responsive and targeted drug delivery. *J. Nanobiotechnology* **2021**, *19*, 1–20, https://doi.org/10.1186/s12951-021-01150-6.

3. da Silva, M.C.; Rocha, C.V.; Bañobre-López, M.; Gallo, J. Stimulation and Suppression of the Innate Immune System through Nanotechnology. *ACS Appl. Nano Mater.* **2021**, *4*, 2303–2316, https://doi.org/10.1021/acsanm.0c03424.
4. Martins, C.D.F.; Raposo, M.M.M.; Costa, S.P.G. A New Fluorogenic Substrate for Granzyme B Based on Fluorescence Resonance Energy Transfer. *Chem. Proc.* **2020**, *3*, 6, https://doi.org/10.3390/ecsoc-24-08311.
5. Gallo, J.; Long, N.J.; Aboagye, E.O. Magnetic nanoparticles as contrast agents in the diagnosis and treatment of cancer. *Chem. Soc. Rev.* **2013**, *42*, 7816–7833, https://doi.org/10.1039/c3cs60149h.
6. Larimer, B.M.; Wehrenberg-Klee, E.; Dubois, F.; Mehta, A.; Kalomeris, T.; Flaherty, K.; Boland, G.; Mahmood, U. Granzyme B PET Imaging as a Predictive Biomarker of Immunotherapy Response. *Cancer Res.* **2017**, *77*, 2318–2327, https://doi.org/10.1158/0008-5472.can-16-3346.

4.3. A View of Medicinal Chemistry through the Looking Glass: Enantioselectivity Studies with Chiral Derivatives of Xanthones

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Over the last few years, the relationship between chirality and biological activity has been of increasing importance in Medicinal Chemistry. Chirality can now be considered as one of the major topics in the design, discovery, development, and marketing of new drugs. The importance of enantioselectivity studies and the increase in chiral drugs in the pharmaceutical market upsurges each year due to the advantages in potency, efficacy, selectivity, and safety associated with the use of single enantiomers. The advances in enantioselective synthesis as well as enantioresolution methodologies aligned to the stricter requirements from regulatory authorities to patent new chiral drug boosted the research in this field [1]. Enantioselectivity studies associated with the biological activity of chiral derivatives of xanthones (CDXs) is an area of great interest of our group [1]. The importance of this class of compounds in Medicinal Chemistry, aligned with the interesting biological and pharmacological activities of some chiral members of this family, the clinical advantages of a single enantiomer over a racemate, and the scarce examples of synthetic CDXs strengthens the obtaining of new synthetic CDXs as single enantiomers.

Herein, some enantioselectivity studies are presented, which demonstrate that the stereochemistry of the CDXs plays a pivotal role in the biological activities, such as tumor cell growth and cyclooxygenases inhibition, P-glycoprotein (P-gp) induction and in virulence effects of resistant bacteria [2,3]. In addition, for each activity, hit compounds were proposed.

To perform that type of study, it is necessary to obtain both enantiomers with very high enantiomeric purity. Liquid chromatography (LC) using chiral stationary phases (CSPs) is the method most widely used to evaluate the enantiomeric purity [2,3]. Moreover, beside the potential as new drugs, CDXs present structural features with interest as chiral selectors for CSPs in LC. Thus, the CDXs were also explored as CSPs for LC [4] and used for the evaluation of enantiomeric purity of new CDXs, achieving enantiomeric ratio values higher than 99%. The analytical application for these small molecules as chiral selectors for LC was discovered by our group for the first time.

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References

1. Fernandes, C.; Carraro, M.L.; Ribeiro, J.; Araújo, J.; Tiritan, M.E.; Pinto, M.M.M. Synthetic Chiral Derivatives of Xanthones: Biological Activities and Enantioselectivity Studies. *Molecules* **2019**, *24*, 791. https://doi.org/10.3390/molecules24040791
2. Pinto, M.M.M.; Palmeira, A.; Fernandes, C.; Resende, D.I.S.P.; Sousa, E.; Cidade, H.; Tiritan, M.E.; Correia-da-Silva, M.; Cravo, S. From Natural Products to New Synthetic Small Molecules: A Journey through the World of Xanthones. *Molecules* **2021**, *26*, 431, https://doi.org/10.3390/molecules26020431.
3. Durães, F.; Cravo, S.; Freitas-Silva, J.; Szemerédi, N.; Martins-Da-Costa, P.; Pinto, E.; Tiritan, M.E.; Spengler, G.; Fernandes, C.; Sousa, E.; et al. Enantioselectivity of Chiral Derivatives of Xanthones in Virulence Effects of Resistant Bacteria. *Pharmaceuticals* **2021**, *14*, 1141, https://doi.org/10.3390/ph14111141.
4. Phyo, Y.Z.; Teixeira, J.; Tiritan, M.E.; Cravo, S.; Palmeira, A.; Gales, L.; Silva, A.M.; Pinto, M.M.; Kijjoa, A.; Fernandes, C. New chiral stationary phases for liquid chromatography based on small molecules: Development, enantioresolution evaluation and chiral recognition mechanisms. *Chirality* **2019**, *32*, 81–97, https://doi.org/10.1002/chir.23142.

5. Oral Presentations

5.1. Synthesis of a High Stokes-Shift Phenylamino–Coumarin Dye with Potential Polarity Sensing Capabilities

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Coumarin derivatives have been widely used for their fluorescence properties in various applications such as fluorescent probes, bioimaging and chemosensors [1,2]. These are highly tunable through synthetic modification, particularly by the inclusion of electron-withdrawing (EWG) and electron-donating groups (EDG) at positions 3 and 7, respectively. Comparatively, there are not many reports of EDGs being employed at position 3 [3,4]. This potential new avenue of photophysical properties prompted us to undertake the synthesis of novel ethynyl-linked dibenziloxycoumarin-aniline dyes (Figure 1), with the key step being the Sonogashira coupling to introduce the tertiary aniline moiety. The prepared dye shows an intense yellow absorption and emission in dichloromethane, revealing an impressive Stokes shift of over 140 nm. Additionally, an increase in solvent polarity leads to a noticeable shift in emission wavelength, making this new core promising as a polarity probe.

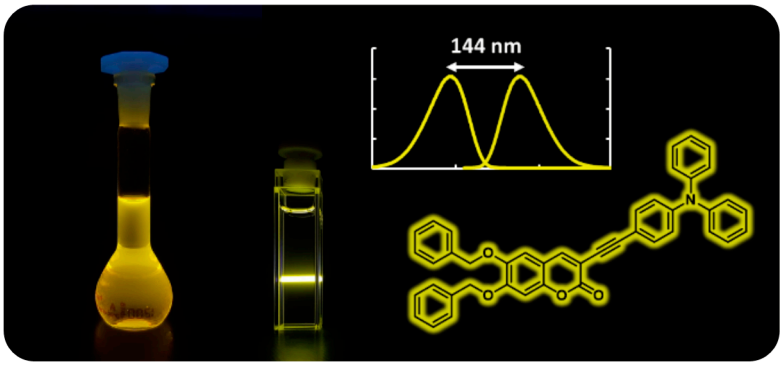

Figure 1. Structure of the coumarin–triphenylamine dye and its respective solution under UV light and with laser irradiation.

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References

1. Mravljak, J.; Ojstersek, T.; Pajk, S.; Dolenc, M. S., Coumarin-based dual fluorescent spin-probes. *Tetrahedron Lett.* **2013**, *54*, 5236–5238, https://doi.org/10.1016/j.tetlet.2013.07.084.
2. Li, H. Q.; Sun, X. Q.; Zheng, T.; Xu, Z. X.; Song, Y. X.; Gu, X. H., Coumarin-based multifunctional chemosensor for arginine/lysine and Cu2+/Al3+ ions and its Cu2+ complex as colorimetric and fluorescent sensor for biothiols. *Sens. Act. B-Chem.* **2019**, *279*, 400–409, https://doi.org/10.1016/j.snb.2018.10.017.
3. Wu, L. L.; Tian, X.; Groleau, R. R.; Wang, J.; Han, H. H.; Reeksting, S. B.; Sedgwick, A. C.; He, X. P.; Bull, S. D.; James, T. D., Coumarin-based fluorescent probe for the rapid detection of peroxynitrite 'AND' biological thiols. *RSC Adv.* **2020**, *10*, 13496–13499, https://doi.org/10.1039/d0ra02234a.
4. Joniak, J.; Stankovicova, H.; Filo, J.; Gaplovska-Kysela, K.; Garaj, V.; Cigan, M., Small-molecule coumarin fluorescent pH probes for extremely acidic conditions. *Sens. Actuator B-Chem.* **2020**, *307*, 10, https://doi.org/10.1016/j.snb.2019.127646.

5.2. New Chiral Spiro-β-Lactams via [3 + 2] Annulation of Allenoates with 6-Alkylidenepenicillanates: Synthesis and Anti-HIV Activity

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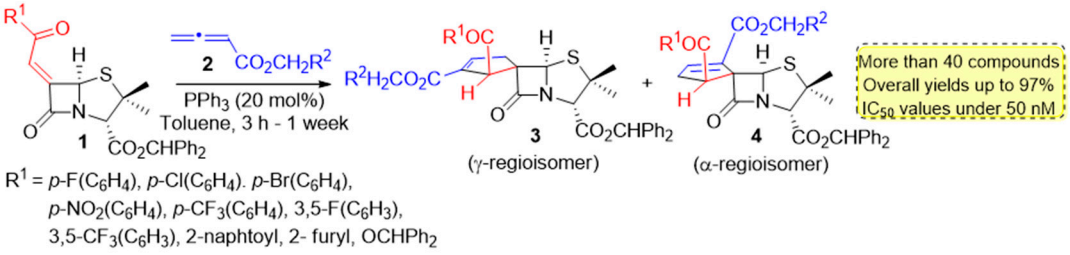
The discovery and development of novel antimicrobial agents with increased bioactivity and stability is a relevant Medicinal Chemistry research goal [1]. Previous studies on the synthesis and biological evaluation of spiro-β-lactams derived from 6-aminopenicillanic acid (6-APA) led us to the discovery of lead compounds with remarkable dual anti-HIV

and anti-Plasmodium properties [2,3]. The identification of this novel class of compounds with potent activity against both infectious agents holds great potential in the fight of both AIDS and malaria.

In this communication, the rational design and synthesis of novel spiro-β-lactams inspired by the previously identified lead compounds will be described. A library of chiral spiro-β-lactams was built by exploring the reactivity of 6-(1-benzoylmethylene)penicillanates **1** as a 2π-component in the formal cycloaddition with allenoates in the presence of PPh_3 (Scheme 1). The synthesized spirocyclic compounds **3** and **4**, derived from 6-alkylidinepenicilanates 1 and benzyl allenoate **2** (R^2 = Ph), were obtained in excellent overall yields (up to 97%). Formal [3+2] cycloaddition reactions were also carried out with benzyl-substituted allenoates **2** (R^2 = *p*-MeC_6H_4, *p*-$MeOC_6H_4$) and with a cinnamyl allenoate **2** (R^2 = CH=CHPh), leading to the corresponding cycloadducts in good yields. The novel spiro-β-lactams were assayed for their in vitro activity against HIV-1, providing relevant structure–activity relationships (SAR). It is noteworthy that this rational design led to the synthesis of six compounds with exceptional anti-HIV activity ($IC_{50} \leq 50$ nM). Remarkably, our best derivative showed an IC_{50} value of 12.37 nM. Further details of this study will be disclosed.



Scheme 1. Synthesis of chiral spiro-β-lactams via [3+2] annulation of allenoates with 6-alkylidenepenicillanates.

Funding: We thank Coimbra Chemistry Centre (CQC), supported by the Portuguese Agency for Scientific Research, "Fundação para a Ciência e a Tecnologia" (FCT) through project UIDB/00313/2020 and UIDP/00313/2020, co-funded by COMPETE2020-UE. iMed. ULisboa, Faculdade de Farmácia de Lisboa, Portugal, is supported by the FCT through Projects UIDB/04138/2020 and UIDP/04138/2020, co-funded by COMPETE2020-UE. Américo J. S. Alves thank the FCT for fellowship SFRH/BD/128910/2017. The authors also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Alves, N.G.; Alves, A.J.S.; Soares, M.I.L.; Pinho e Melo, T.M.V.D. Recent Advances in the Synthesis of Spiro-β-Lactams and Spiro-δ-Lactams. *Adv. Synth. Catal.* **2021**, *363*, 2464–2501, https://doi.org/10.1002/adsc.202100013.
2. Bártolo, I.; Santos, B.S.; Fontinha, D.; Machado, M.; Francisco, D.; Sepodes, B.; Rocha, J.; Mota-Filipe, H.; Pinto, R.; Figueira, M.E.; et al. Spiro-β-lactam BSS-730A Displays Potent Activity against HIV and Plasmodium. *ACS Infect. Dis.* **2021**, *7*, 421–434, https://doi.org/10.1021/acsinfecdis.0c00768.
3. Alves, N.G.; Bártolo, I.; Alves, A.J.; Fontinha, D.; Francisco, D.; Lopes, S.M.; Soares, M.I.; Simões, C.J.; Prudêncio, M.; Taveira, N.; et al. Synthesis and structure-activity relationships of new chiral spiro-β-lactams highly active against HIV-1 and Plasmodium. *Eur. J. Med. Chem.* **2021**, *219*, 113439, https://doi.org/10.1016/j.ejmech.2021.113439.

5.3. Synthesis of New Aminocyclopentenes via Photoflow of Pyridinium Salts Followed by Pd Catalysis

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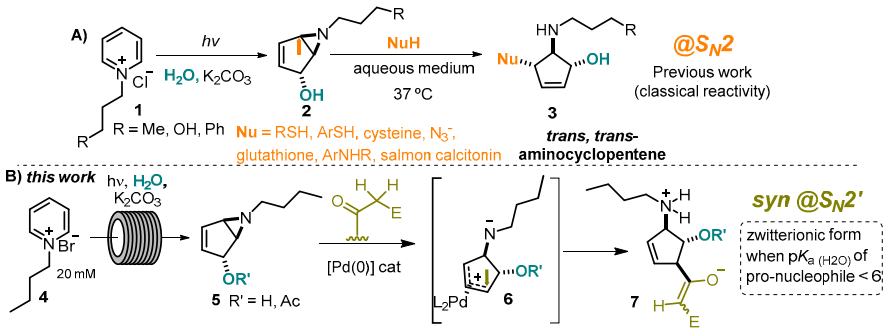
In 1972, Kaplan et al. reported a pioneer study in the photochemical transformation of *N*-methylpyridinium chlorides to 6-methylazabicyclo[3.1.0]hex-3-en-2-ols (bicyclic aziridine) [1]. In 2016, we described the photoreaction of several pyridinium salts **1** into the corresponding bicyclic aziridines **2** under batch conditions with low productivity. S_N2 ring-opening reaction of **2** in water with heteroatom-based nucleophiles allowed the synthesis of new *trans*, *trans*-aminocyclopentenes (Scheme 1A) [2]. This powerful photocyclization, stereo and regioselective S_N2 aziridine ring-opening sequence was applied to the total synthesis of several natural and non-natural products, such as (+)-mannostatin A and (+)-castanospermine [3,4]. Here, we will present the application of flow on the photoreaction of *N*-butyl pyridinium salt **4** (Scheme 1B), allowing to solve the scalability problem of this rearrangement and consequently producing bicyclic aziridine **5** on a gram scale [5,6]. Furthermore, the palladium-catalyzed ring opening of bicyclic aziridine **5** with active methylenes (Scheme 1B) presented a new selectivity due to η^3-allylpalladium complex formation and nucleophile addition anti to the allylic oxy group **6**, resulting in new aminocyclopentenes **7** [7].



Scheme 1. (**A**) Photoreaction of pyridinium salt followed by classical SN2 aziridine ring opening reaction. (**B**) Photoflow of pyridinium salts and palladium catalyzed aziridine ring-opening reaction.

Funding: We thank the Fundação para a Ciência e Tecnologia for financial support (PhD grant 2020.04589.BD) and project (PTDC/QUI-QOR/32008/2017, UIDB/04138/2020, UIDP/04138/2020 and PESSOA 2018/2019 (Proc. 441.00 França and PHC PESSOA 2018 No 40875QJ)).The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 951996.

References

1. Kaplan, L.; Pavlik, J.W.; Wilzbach, K.E. Photohydration of pyridinium ions. *J. Am. Chem. Soc.* **1972**, *94*, 3283–3284, https://doi.org/10.1021/ja00764a089.
2. Vale, J.R.; Siopa, F.; Branco, P.S.; Afonso, C.A.M. Ring Opening of 6-Azabicyclo [3.1.0]hex-3-en-2-ols in Water under Mild Conditions. *Eur. J. Org Chem.* **2016**, *2016*, 2048–2053, https://doi.org/10.1002/ejoc.201501468.

3. Damiano, T.; Morton, D.; Nelson, A. Photochemical transformations of pyridinium salts: mechanistic studies and applications in synthesis. *Org. Biomol. Chem.* **2007**, *5*, 2735–2752, https://doi.org/10.1039/b706244n.
4. Zou, J.; Mariano, P.S. The synthetic potential of pyridinium salt photochemistry. *Photochem. Photobiol. Sci.* **2008**, *7*, 393–404, https://doi.org/10.1039/b801808c.
5. Siopa, F.; António, J.P.M.; Afonso, C.A.M. Flow-Assisted Synthesis of Bicyclic Aziridines *via* Photochemical Transformation of Pyridinium Salts. *Org. Process Res. Dev.* **2018**, *22*, 551–556, https://doi.org/10.1021/acs.oprd.8b00036.
6. Fortunato, M.A.G.; Ly, C.-P.; Siopa, F.; Afonso, C.A.M. Process Intensification for the Synthesis of 6-Allyl-6-azabicyclo[3.1.0]hex-3-en-2-ol from 1-Allylpyridinium Salt Using a Continuous UV-Light Photoflow Approach. *Methods Protoc.* **2019**, *2*, 67, https://doi.org/10.3390/mps2030067.
7. Oliveira, J.A.; Kiala, G.; Siopa, F.; Bernard, A.; Gontard, G.; Oble, J.; Afonso, C.A.; Poli, G. Palladium-catalyzed allylic substitution between C-based nucleophiles and 6-azabicyclo[3.1.0]-hex-3-en-2-oxy derivatives: A new selectivity paradigm. *Tetrahedron* **2020**, *76*, 131182, https://doi.org/10.1016/j.tet.2020.131182.

5.4. Reactivity between Cork and Wine Compounds

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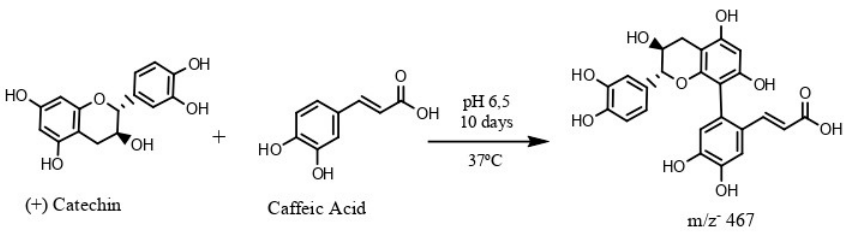
Different phenolic compounds have been found to be extracted from cork stoppers into bottled wine model solutions. Some of these compounds were found in large quantities including gallic acid, protocatechuic acid, protocatechuic aldehyde, caffeic acid, vanillin, sinapic acid, ferulic acid and ellagic acid [1]. It has been demonstrated that these compounds have an impact in the color and taste of wines [2]. On the other hand, some of them participate in polymerization reactions with some wine components, changing their sensorial properties and redox status [3,4]. In addition, it is also known that phenolic acids and aldehydes can react resulting in more complex structures found in aged wines [5,6], which affect some chromatic qualities.

With this in mind, the aim of this study was to evaluate the reactivity between cork compounds and wine components, namely catechin, and to understand the impact of them on the wine properties. The reaction of several phenolic acids with (+)-catechin was studied in a wine model solution. After 4 months, at pH 3.2, the formation of a compound with an ion mass (in the negative ion mode) at m/z 467 was observed with the proposed structure for the obtained compound presented in Scheme 1.



Scheme 1. Reaction scheme and structure proposed for the obtained compound.

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References

1. Azevedo, J.; Fernandes, I.; Lopes, P.; Roseira, I.; Cabral, M.; Mateus, N.; Freitas, V. Migration of phenolic compounds from different cork stoppers to wine model solutions: antioxidant and biological relevance. *Food Res. Technol.* **2014**, *239*, 951–960, https://doi.org/10.1007/s00217-014-2292-y.
2. Glabasnia, A.; Hofmann, T. Sensory-Directed Identification of Taste-Active Ellagitannins in American (Quercus alba L.) and European Oak Wood (Quercus robur L.) and Quantitative Analysis in Bourbon Whiskey and Oak-Matured Red Wines. *J. Agric. Food Chem.* **2006**, *54*, 3380–3390, https://doi.org/10.1021/jf052617b.
3. Chassaing, S.; Lefeuvre, D.; Jacquet, R.; Jourdes, M.; Ducasse, L.; Galland, S.; Grelard, A.; Saucier, C.; Teissedre, P.-L.; Dangles, O.; Quideau, S. Physicochemical Studies of New Anthocyano-Ellagitannin Hybrid Pigments: About the Origin of the Influence of Oak C-Glycosidic Ellagitannins on Wine Color. *Eur. J. Org. Chem.* **2010**, *2010*, 55–63, https://doi.org/10.1002/ejoc.200901133.
4. Quideau, S.; Jourdes, M.; Lefeuvre, D.; Montaudon, D.; Saucier, C.; Glories, Y.; Pardon, P.; Pourquier, P. The Chemistry of Wine PolyphenolicC-Glycosidic Ellagitannins Targeting Human Topoisomerase II. *Chem. A Eur. J.* **2005**, *11*, 6503–6513, https://doi.org/10.1002/chem.200500428.
5. Schwarz, M.; Jerz, G.; Winterhalter, P. Isolation and structure of Pinotin A, a new anthocyanin derivative from Pinotage wine. *Vitis* **2003**, *42*, 105–106, https://doi.org/10.5073/vitis.2003.42.105-106.
6. Es-Safi, N.-E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Competition between (+)-Catechin and (−)-Epicatechin in Acetaldehyde-Induced Polymerization of Flavanols. *J. Agric. Food Chem.* **1999**, *47*, 2088–2095, https://doi.org/10.1021/jf980628h.

5.5. Overcoming β-Lactam Resistance in MRSA: Searching for Hit Compounds for PBP2a Inhibition

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital- and community-acquired infections worldwide, with a high mortality rate due to resistance to most β-lactam antibiotics [1,2]. MRSA resistance is related to the acquisition of a PBP2a-coding *mecA* gene. The protein coded by this gene has low affinity to β-lactams, meaning that cell wall peptidoglycan formation is not blocked, and bacteria survive [1,2]. *S. aureus* PBP2a adopts a closed active site conformation, inaccessible for β-lactams, which can efficiently perform peptidoglycan crosslinking. This reduced PBP2a susceptibility to β-lactams is related to both conformational changes at a serine nucleophile in the active site, resulting in high affinity toward the substrate but reduced affinity for β-lactams and to the presence of a loop protecting the active site from inhibitors. The conformational change of this loop is regulated by an allosteric site distal from the active site, which becomes accessible only when the allosteric site is occupied [3]. Thus, there is a pressing need for innovative antibiotics to overcome resistance in these strains.

In this work, a structure-based computational molecular docking screening approach was applied with Autodock Vina, using the X-ray structures of the target protein in the closed and open conformations (PDB ID 1vqq and 3zg0, respectively). Several β-lactam compounds and fluorenone derivatives were tested as possible inhibitors for both catalytic and allosteric sites. Known specific inhibitors were used as controls.

The known inhibitor, L-695256, with the best results to the target protein presented affinities of −6.2 kcal mol^{-1} for the allosteric site in the native PBP2a and -9.4 kcal.mol^{-1}

for the active site of acylated PBP2a protein (PDB:3zg0). The promising hit compounds tested in this work presented significant improvements in affinity for both catalytic sites: for instance, −7.3 kcal mol^{-1} for the allosteric site in the native PBP2a (PDB:1vqq) and −11 kcal mol^{-1} for the active site of acylated PBP2a protein (PDB:3zg0). These obtained lower binding energies correspond to more favorable ligand–protein interactions. Moreover, hit compounds that bind to the same residues have known inhibitors, maintaining the expected interactions to the protein.

These results indicate that tested compounds are promising hits targeting both catalytic sites of PBP2a protein from MRSA, contributing toward their potential use to overcome β-lactam resistance. Molecular dynamics simulations using GROMACS software are currently being deployed, aiming to understand whether the binding of the hit compounds to the allosteric site can induce protein conformational change, contributing to a more accessible active site.

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References

1. Ferrer-González, E.; Kaul, M.; Parhi, A.J.; LaVoie, E.J.; Pilcha, D.S. ß-Lactam antibiotics with a high affinity for pbp2 act synergistically with the ftsz-targeting agent txa707 against methicillin-resistant staphylococcus aureus. *Antimicrob. Agents Chemother.* **2017**, *61*, e00863-17, https://doi.org/10.1128/AAC.00863-17.
2. Lim, D.; Strynadka, N.C. Structural basis for the β lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. *Nat. Genet.* **2002**, *9*, 870–876, https://doi.org/10.1038/nsb858.
3. Mahasenan, K.V.; Molina, R.; Bouley, R.; Batuecas, M.T.; Fisher, J.F.; Hermoso, J.A.; Chang, M.; Mobashery, S. Conformational dynamics in penicillin-binding protein 2a of methicillin-resistant staphylococcus aureus, allosteric communication network and enablement of catalysis. *J. Am. Chem. Soc.* **2017**, *139*, 2102–2110, https://doi.org/10.1021/jacs.6b12565.

5.6. Betulinic Acid as Raw Material to Produce Novel Added-Value Compounds

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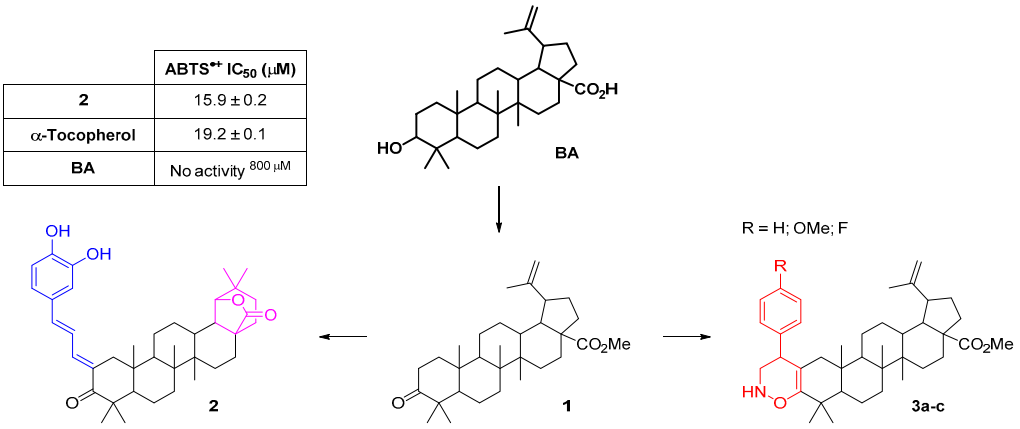
Betulinic acid (BA), a lupane-type pentacyclic triterpenic acid, is commonly isolated from the bark of birch trees (*Betula* spp.), displaying important biological activities [1,2], and an attractive scaffold for chemical upgrading [3]. Aiming at valorizing biomass derived compounds, our main goal was to decorate BA with well-known bioactive moieties (e.g., catechol, 1,2,3-triazole, pyridine, among others) in order to obtain hybrid compounds with enhanced biological properties.

In this context, we have been interested in synthesizing polyhydroxylated BA-based compounds as new amphiphilic antioxidants [4]. Indeed, the 19,28-epoxyoleanane-3,28-dione-type derivative **2**, bearing a catechol moiety and an extended π-conjugated carbonyl

system (Scheme 1), emerged as lead compound, since it was revealed to be the most efficient scavenger for $ABTS^{\bullet+}$, being more active than the pristine BA and α-tocopherol (used as a positive control). Moreover, we have also been interested in establishing a stepwise methodology for the functionalization of BA with oxygen and nitrogen heterocycles such as 2*H*-1,2-oxazine, 4*H*-pyran, pyridine and 1,2,3-triazole. To the best of our knowledge, it is the first time that a 2*H*-1,2-oxazine ring, which is a six-membered *N*,*O*-heterocycle, was installed in the BA skeleton **3a–c** (Scheme 1).

	$ABTS^{\bullet+}$ IC_{50} (μM)
2	15.9 ± 0.2
α-Tocopherol	19.2 ± 0.1
BA	No activity $^{800\ \mu M}$



Scheme 1. Synthetic analysis of the most efficient oleanane-type antioxidant **2** and BA-based hybrids containing a 2*H*-1,2-oxazine ring **3a–c**.

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References

1. Moghaddam, M.G.; Ahmad, F.B.H.; Samzadeh-Kermani, A. Biological Activity of Betulinic Acid: A Review. *Pharmacol. Pharm.* **2012**, *3*, 119–123, https://doi.org/10.4236/pp.2012.32018.
2. Ríos, J.L.; Máñez, S. New Pharmacological Opportunities for Betulinic Acid. *Planta Med.* **2018**, *84*, 8–19, https://doi.org/10.1055/s-0043-123472.
3. Sousa, J.L.C.; Freire, C.S.R.; Silvestre, A.J.D.; Silva, A.M.S. Recent Developments in the Functionalization of Betulinic Acid and Its Natural Analogues: A Route to New Bioactive Compounds. *Molecules* **2019**, *24*, 355.
4. Sousa, J.L.C.; Gonçalves, C.; Ferreira, R.M.; Cardoso, S.M.; Freire, C.S.R.; Silvestre, A.J.D.; Silva, A.M.S. Functionalization of Betulinic Acid with Polyphenolic Fragments for the Development of New Amphiphilic Antioxidants. *Antioxidants* **2021**, *10*, 148.

5.7. Sulfonamide Porphyrins as Antimicrobial Photosensitizers: The Role of Co-Adjuvants

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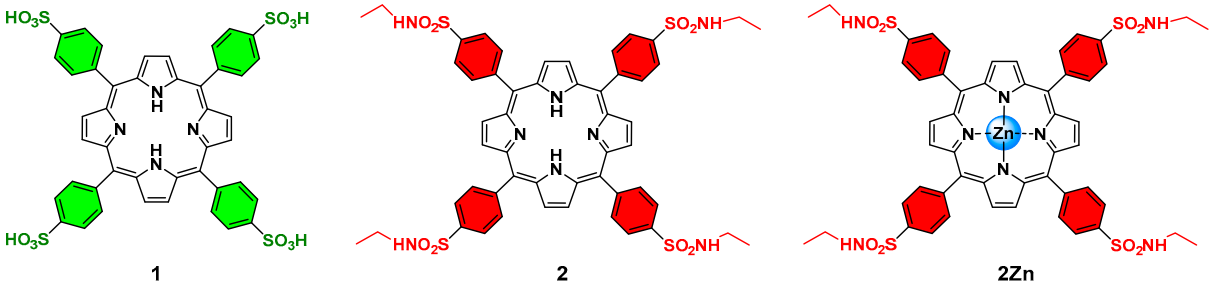
Sulfonamides or sulfa drugs were the first effective synthetic drugs used systematically against a broad spectrum of bacteria [1]. Since their discovery in 1935 [2], microbial resistance to conventional antimicrobials has been a major concern, and recent strategies have focused on the development of novel treatment options and alternative antimicrobial therapies. Antimicrobial Photodynamic Therapy (aPDT) [3] involves the combination of photoactive dyes and harmless visible light to produce reactive oxygen species (ROS) that can selectively kill microbial cells. This therapeutic modality is being recognized as an effective method to inactivate a broad spectrum of microorganisms, including those resistant to conventional antimicrobials/biocides. In this communication, the development of porphyrins (**1**, **2**) and a metalloporphyrin, (**2Zn**) functionalized with sulfonamides and sulfonic acids [4], (Scheme 1) and their efficacy to generate reactive oxygen species and to photoinactivate microorganisms, was reported.



Scheme 1. The three photosensitizers tested proved to be efficient in eradicating methicillin-resistant *Staphylococcus aureus* (MRSA), highlighting 5,10,15,20-tetrakis(4-sulfophenyl)porphyrin (**1**). The combination with co-adjuvants improved the photodynamic effect of the three photosensitizers, allowing us to reduce the irradiation time as well as the concentration of the used photosensitizer.

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References

1. Haller, J.S. The First Miracle Drugs: How the Sulfa Drugs Transformed Medicine (review). *J. Hist. Med. Allied Sci.* **2008**, *63*, 119–121.
2. Feng, M.; Tang, B.; Liang, S.H.; Jiang, X. Sulfur Containing Scaffolds in Drugs: Synthesis and Application in Medicinal Chemistry. *Curr. Top. Med. Chem.* **2016**, *16*, 1200–1216, https://doi.org/10.2174/1568026615666150915111741.
3. Mesquita, M.Q.; Dias, C.J.; Neves, M.G.P.M.S.; Almeida, A.; Faustino, M.A.F. Revisiting Current Photoactive Materials for Antimicrobial Photodynamic Therapy. *Molecules* **2018**, *23*, 2424.
4. Monteiro, C.J.P.; Pereira, M.M.; Pinto, S.M.A.; Simões, A.V.C.; Sá, G.F.F.; Arnaut, L.G.; Formosinho, S.J.; Simões, S.; Wyatt, M.F. Synthesis of amphiphilic sulfonamide halogenated porphyrins: MALDI-TOFMS characterization and evaluation of 1-octanol/water partition coefficients. *Tetrahedron* **2008**, *64*, 5132–5138.

5.8. From Lab Bench to Market: A Long Journey to the Development of a Medicine

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Each year, several new medicines are approved for human use. The journey to the development of a new medicine begins with the identification, selection and validation of a target, confirming its critical role in the disease modulation. During the drug discovery phase, thousands of compounds can be considered, but only few (with sufficient and relevant data) can be selected to proceed. These initial molecules will progress to pre-clinical studies where extensive laboratory and animal experiments are conducted to determine their safety for the next phase: clinical trials in humans.

Along this entire development, the contribution of chemistry is not confined to the drug discovery stage. The pre-clinical and clinical stages involve increasing amounts (grams to kilograms scale) of the candidate compound(s). In this context, detailed studies need to be conducted by process chemists in order to evaluate the feasibility of scaling up a chemical process as well as to improve and optimize the synthesis pathway with the aim of ensuring quality, safety and reproducibility. This work is crucial for the human clinical trials, where the compounds are manufactured under strict Good Manufacturing Practices (GMP) standards to ensure they meet the quality requirements according to regulatory guidelines.

The development of a new medicine is a complex process; it requires a strong inter-disciplinary teamwork, and its progression to the market will only be approved when considered safe and effective for its intended use.

5.9. Synthesis of Chiral 2-(Tetrazol-5-yl)-2H-Azirines: An Approach to Molecules with Relevance in Medicinal Chemistry

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Antimicrobial resistance is a global threat of rising concern to human and animal health [1]. Unfortunately, the development of new antimicrobial drugs has been scarce over the past years, making it more difficult to treat resistant bacterial and fungal infections. Nature has been a major source of biologically active compounds; for instance, naturally occurring 2*H*-azirine-2-carboxylates azirinomycin, (-)-dysidazirine and motualevic acid F, exhibit biological activity against bacteria and fungi [2–6]. Recently, we described the one-pot asymmetric Neber reaction of β-ketoxime tetrazole derivatives (e.g., **1**) leading to 2-(tetrazol-5-yl)-2*H*-azirines (e.g., **2**) resorting to organocatalysis (Figure 1) [4–6]. Further studies on the symmetric synthesis of new chiral 2-(tetrazol-5-yl)-2*H*-azirines as well as our efforts to apply this methodology to the synthesis of bioisosteres of the aforementioned biologically active 2*H*-azirine-2-carboxylates were discussed.

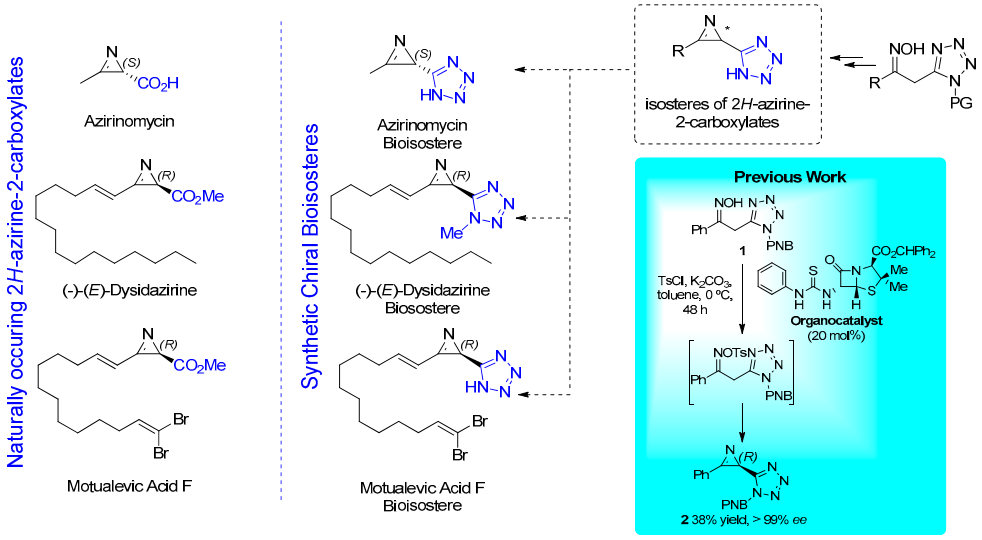

Figure 1. Natural occurring and new chiral 2-(tetrazol-5-yl)-2*H*-azirine bioisosteres.

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References

1. Forsberg, K.; Woodworth, K.; Walters, M.; Berkow, E.L.; Jackson, B.; Chiller, T.; Vallabhaneni, S. Candida auris: The recent emergence of a multidrug-resistant fungal pathogen. *Med. Mycol.* **2019**, *57*, 1–12, https://doi.org/10.1093/mmy/myy054.
2. Miller, T.W.; Tristram, E.W.; Wolf, F.J. Azirinomycin. II. Isolation and chemical characterization as 3-methyl-2(2H) azirinecarboxylic acid. *J. Antibiot.* **1971**, *48*, 48–50.
3. Molinski, T.F.; Ireland, C.M. Dysidazirine, a cytotoxic azacyclopropene from the marine sponge Dysidea fragilis. *J. Org. Chem.* **1988**, *53*, 2103–2105, https://doi.org/10.1021/jo00244a049.
4. Cardoso, A.L.; Gimeno, L.; Lemos, A.; Palacios, F.; e Melo, T.M.V.D.P. The Neber Approach to 2-(Tetrazol-5-yl)-2H-Azirines. *J. Org. Chem.* **2013**, *78*, 6983–6991, https://doi.org/10.1021/jo4006552.
5. Alves, C.C., Asymmetric Neber reaction in the synthesis of 2-(tetrazol-5-yl)-2H-azirines. Master's Thesis, Universidade de Coimbra, Coimbra, Portugal, 2018.
6. Alves, C.; Grosso, C.; Barrulas, P.; Paixão, J.A.; Cardoso, A.L.; Burke, A.J.; Lemos, A.; e Melo, T.M.V.D.P. Asymmetric Neber Reaction in the Synthesis of Chiral 2-(Tetrazol-5-yl)-2H-Azirines. *Synlett* **2020**, *31*, 553–558, https://doi.org/10.1055/s-0039-1691533.

5.10. A Multiomics Approach in the Study of Montelukast Neurotoxicity

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Montelukast (MTK) is a cysteinyl leukotriene receptor antagonist widely used to suppress the inflammatory response in asthma and allergic rhinitis. Despite being suggested as a potential therapeutic strategy for neuroinflammatory disorders, such as Alzheimer's disease, the number of reported adverse drug reactions (ADRs), among which neuropsychiatric ADRs are the most reported, has been increasing.

Considering the unexplained neurotoxicity of MTK and its potential repurposing applications, our goal was to understand the influence of MTK on the metabolome and proteome using a multiomics approach. Test mice were treated with 1 mg/kg MTK by oral gavage once a day for one week, and selected tissues and fluids were collected for metabolite and protein extraction. Samples were analyzed by ultra-performance liquid chromatography coupled to high-resolution electrospray ionization tandem mass spectrometry (UPLC-ESI-HRMS/MS), which was followed by a bioinformatics-driven data treatment approach using XCMS [1] and MaxQuant [2].

Brain metabolomics data show that pathways involved in energy management and in the turnover of biomolecular building blocks (such as amino acids and nucleosides) are the ones most affected by montelukast. Neurosteroid and neuromodulator pathways were also affected. An increased corticosterone level suggests a hyper-activated neuroendocrine system, which is involved in stress response, mood changes, and emotion. The hypothesis of hypothalamic–pituitary–adrenal axis (HPA) hyper-activation is supported by an increase in serotonin and histamine levels. Other neurotransmitters with important roles in brain processes were also affected by MTK treatment, namely dopamine. Species involved in redox maintenance were also unbalanced. A decrease in the GSH/GSSG ratio suggests an increased global oxidative stress, which could be implicated in some brain disorders. Decreased L-γ-glutamylcysteine and dihydrolipoate levels also point to a redox unbalance, with an overall increased oxidative status. This oxidative stress hypothesis is consistent with our previous data obtained in metabolism studies, where a non-enzymatic MTK–GSH conjugate was identified. Furthermore, proteomics data suggest that MTK administration influences endopeptidase activity, apoptotic processes, and cell death.

To conclude, the network connectivity between both omics approaches supports the relation between metabolites and proteins in pathways influenced by montelukast.

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References

1. Tautenhahn, R.; Patti, G. J.; Rinehart, D.; Siuzdak, G., XCMS Online: a web-based platform to process untargeted metabolomic data. *Anal. Chem.* **2012**, *84*, 5035–5039.
2. Tyanova, S.; Temu, T.; Sinitcyn, P.; Carlson, A.; Hein, M. Y.; Geiger, T.; Mann, M.; Cox, J., The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat. Methods* **2016**, *13*, 731–740.

5.11. Bis-Furans: A Sustainable Source of Structurally Diverse Scaffolds

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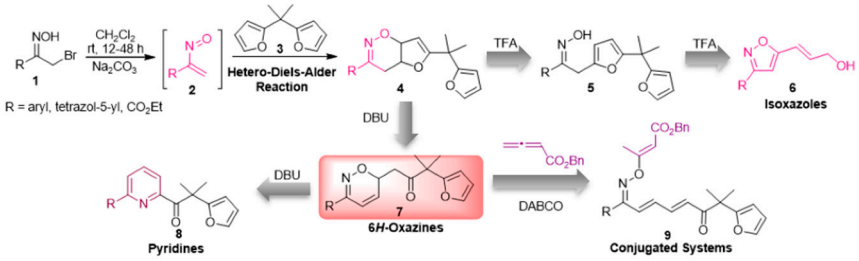
Due to its rich chemistry, furan is an important scaffold in organic synthesis and has been explored as a building block in the construction of a wide range of heterocyclic and acyclic structures, some of which are finding applications in natural product synthesis and Medicinal Chemistry [1,2]. Moreover, furan is a biomass-platform molecule being easily prepared by the decarbonylation of furfural. The hetero-Diels–Alder reaction between

conjugated nitroso- and azoalkenes and electron-rich heterocycles has been one of our topics of research [3–5]. In this context, we have described the dienophilic behavior of 2,2-difuran-2-ylpropane (3) toward nitroso- (e.g., 2) and azoalkenes, generated in situ by base-mediated dehydrohalogenation of α-halo oximes (e.g., 1) and α-halo hydrazones, respectively, giving access to a great variety of dihydrofurooxazines (e.g., 4) and tetrahydropyridazines (Scheme 1) [6,7]. In this communication, studies on the reactivity of these dihydrofurooxazines 4 to originate functionalized heterocyclic and acyclic compounds will be discussed. The acid- and base-catalyzed rearrangement of cycloadducts 4 and analogues gave rise to the formation bis-furans 5 bearing an open-chain oxime and 6H-oxazines 7, respectively. Bis-furans 5 upon treatment with TFA underwent further transformations via interesting rearrangements affording isoxazoles 6, whereas the DBU-promoted rearrangement of 6H-oxazines 7 leads to pyridines 8. In addition, 6H-oxazines 7 were efficiently converted into the unexpected conjugated systems 9 by DABCO-mediated reaction with allenes. In this communication, details of this study and the mechanisms underlying these transformations were disclosed.



Scheme 1. Exploring the chemistry of bis-furans.

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References

1. Makarov, A.S.; Merkushev, A.A.; Uchuskin, M.G.; Trushkov, I.V. Oxidative Furan-to-Indole Rearrangement. Synthesis of 2-(2-Acylvinyl)indoles and Flinderole C Analogues. *Org. Lett.* **2016**, *18*, 2192–2195.
2. Kalaitzakis, D.; Triantafyllakis, M.; Sofiadis, M.; Noutsias, D.; Vassilikogiannakis, G. Photooxygenation of Furylalkylamines: Easy acess to Pyrrolizidine and Indolizidine Scaffolds. *Angew. Chem. Int. Ed.* **2016**, *55*, 4605–4609.
3. Grosso, C.; Cardoso, A.L.; Lemos, A.; Varela, J.; Rodrigues, M.J.; Custódio, L.; Barreira, L.; Pinho e Melo, T.M.V.D. Novel approach to bis(indolyl)methanes: de novo synthesis of 1-hydroxyiminomethyl derivatives. *Eur. J. Med. Chem.* **2015**, *93*, 9–15.
4. Grosso, C.; Cardoso, A.L.; Rodrigues, M.J.; Marques, C.; Barreira, L.; Lemos, A.; Pinho e Melo, T.M.V.D. Hetero-Diels-Alder approach to Bis(indolyl)methanes. *Bioorg. Med. Chem.* **2017**, *25*, 1122–1131.
5. Lopes, S.M.M.; Cardoso, A.L; Lemos, A.; Pinho e Melo, T.M.V.D. Recent Advances in the Chemistry of Conjugated Nitrosoalkenes and Azoalkenes. *Chem. Rev.* **2018**, *118*, 11324–11352.
6. Lopes, S.M.M.; Henriques, M.S.C.; Paixão, J.A.; Pinho e Melo, T.M.V.D. Exploring the Chemistry of Furans: Synthesis of Functionalized Bis(furan-2-yl)methanes and 1,6-Dihydropyridazines. *Eur. J. Org. Chem.* **2015**, *2015*, 6146–6151.
7. Alves, A.J.S.; Lopes, S.M.M.; Henriques, M.S.C.; Paixão, J.A.; Pinho e Melo, T.M.V.D. Hetero-Diels-Alder and Ring-Opening Reactions of Furans applied to the Synthesis of Functionalized Heterocycles. *Eur. J. Org. Chem.* **2017**, *2017*, 4011–4025.

5.12. Phosphate Prodrug Technology: Pursuing a New Therapeutic Approach for GNE Myopathy

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ProTide technology employs phosphate-masking groups capable of providing more favorable drug-like properties to a substrate to which it is attached. This technology has been successfully applied to generate several new medicines, and more recently, N-acetyl-D-mannosamine (ManNAc) phosphoramidate prodrugs have been developed for the potential substrate replacement treatment for GNE myopathy (GNEM) [1]. ManNAc phosphoramidate prodrugs may bypass the deficient GNE enzyme observed in GNEM patients [2] by directly providing a source of intracellular ManNAc-6-P (Figure 1).

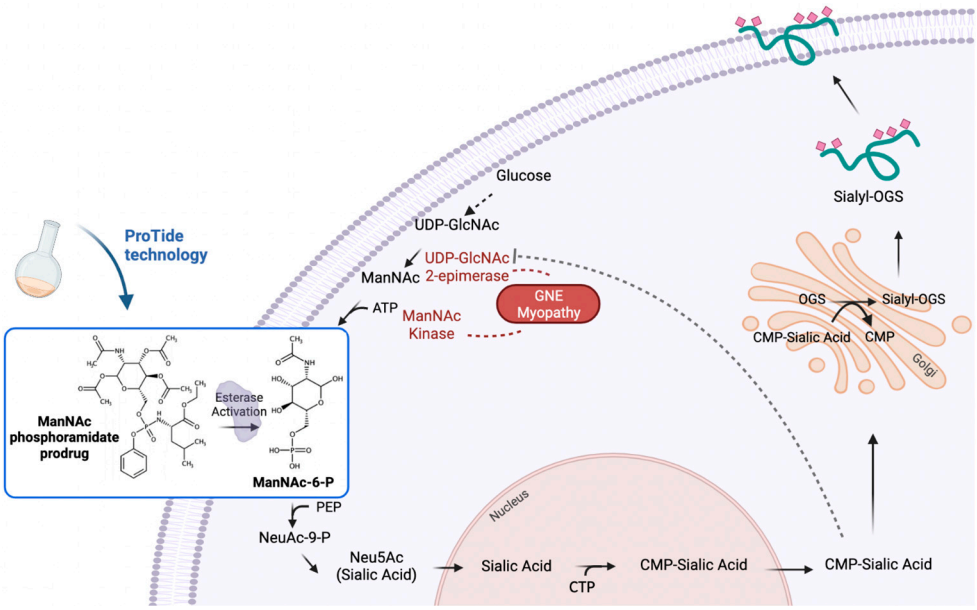

Figure 1. Schematic representation of the proposed therapeutic strategy by ManNAc phosphoramidate prodrugs in sialic acid biosynthetic pathway.

Herein, we aimed at predicting pharmacokinetic properties of a small library of prodrugs, using computer-aided drug design (CADD) methodologies. Prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) was carried out using the web server pkCSM. For drug-likeness, chemical descriptors, such as the octanol–water parti-

tion coefficient (LogP), and the topological polar surface area (TPSA) were retrieved from SwissADME software.

When compared with ManNAc, currently in phase II clinical trial, all prodrugs showed better lipid solubility (higher LogP) that may help them to interact with cell membranes. According to our model, all prodrugs are predicted to be substrates of CYP3A4, which is responsible for the metabolism of more than 50% of drugs, suggesting they may be extensively metabolized in the liver. Therefore, their hepatotoxic potential cannot be set aside, and docking studies are being conducted to understand the interaction with hepatic enzymes.

This work will help guide the selection of lead candidates to proceed to in vivo studies, using animal models that recapitulate GNEM.

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References

1. Morrozi, C.; Sedláková, J.; Serpi, M.; Avigliano, M.; Carbajo, R.; Sandoval, L.; Valles-Ayoub, Y.; Crutcher, P.; Thomas, S.; Pertusati, F. Targeting GNE myopathy: a dual prodrug approach for the delivery of N-acetylmannosamine 6-phosphate. *J. Med. Chem.* **2019**, *62*, 8178–8193.
2. Pogoryelova, O.; Coraspe, J.A.G.; Nikolenko, N.; Lochmüller, H.; Roos, A. GNE myopathy: from clinics and genetics to pathology and research strategies. *Orphanet J. Rare Dis.* **2018**, *13*, 70.

5.13. Highway to Bioactivity: Isatin-Based Multicomponent Reactions

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Multicomponent reactions (MCRs) are a sustainable and efficient way to achieve structural diversity, and therefore, their application in drug discovery has been gaining momentum over recent years [1,2]. Using privileged scaffolds, such as isatin, as a starting point for MCRs proves to be a valuable approach to generate druglike libraries of compounds with promising biological activity [3,4].

Herein, we report our recent efforts in engaging isatin in the Ugi four-component reaction (U4CR) and Ugi four-center three-component reaction (U4c3CR), as well as isatin's conversion to another valuable scaffold in Medicinal Chemistry, tryptanthrin, and the application of this alkaloid in the Petasis three-component reaction (P3CR) (Figure 1) [5–7].

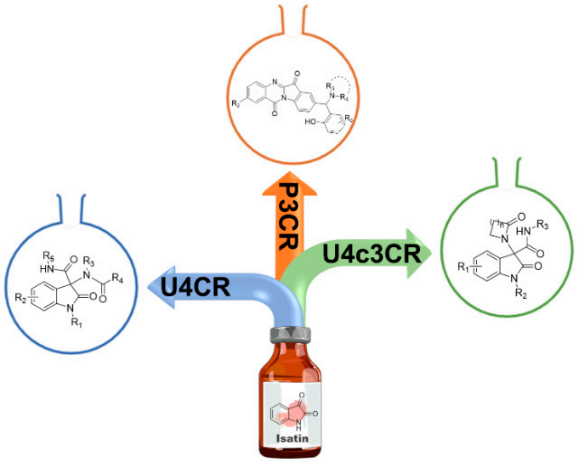

Figure 1. Isatin as a starting point for MCRs.

Funding: We thank PhD fellowship PD/BD/128490/2017 of the FCT PhD Programme CATSUS (PD/00248/2012) and Projects/Grants: UIDB/00313/2020, UIDP/00313/2020, REEQ/481/QUI/2006, RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012, UIDB/50006/2020 (from FCT/FEDER/COMPETE).

References

1. Cioc, R.C.; Ruijter, E.; Orru, R.V.A. Multicomponent reactions: advanced tools for sustainable organic synthesis. *Green Chem.* **2014**, *16*, 2958–2975, https://doi.org/10.1039/c4gc00013g.
2. Ruijter, E.; Orru, R.V. Multicomponent reactions – opportunities for the pharmaceutical industry. *Drug Discov. Today Technol.* **2013**, *10*, e15–e20, https://doi.org/10.1016/j.ddtec.2012.10.012.
3. Brandão, P.; Marques, C.S.; Carreiro, E.P.; Pineiro, M.; Burke, A.J. Engaging Isatins in Multicomponent Reactions (MCRs) Easy Access to Structural Diversity. *Chem. Rec.* **2021**, *21*, 924–1037, https://doi.org/10.1002/tcr.202000167.
4. Brandão, P.; Marques, C.; Burke, A.J.; Pineiro, M. The application of isatin-based multicomponent-reactions in the quest for new bioactive and druglike molecules. *Eur. J. Med. Chem.* **2020**, *211*, 113102, https://doi.org/10.1016/j.ejmech.2020.113102.
5. Brandão, P.; Puerta, A.; Padrón, J.M.; Kuznetsov, M.L.; Burke, A.J.; Pineiro, M. Ugi Adducts of Isatin as Promising Antiproliferative Agents with Druglike Properties. *Asian J. Org. Chem.* **2021**, *10*, 3434–3455, https://doi.org/10.1002/ajoc.202100684.
6. Brandão, P.; López, Ó.; Leitzbach, L.; Stark, H.; Fernández-Bolaños, J. G.; Burke, A. J.; Pineiro, M. Ugi Reaction Synthesis of Oxindole–Lactam Hybrids as Selective Butyrylcholinesterase Inhibitors. *ACS Med. Chem. Lett.* **2021**, *12*, 1718–1725, https://doi.org/10.1021/acsmedchemlett.1c00344.
7. Brandão, P.; Marques, C.; Pinto, E.; Pineiro, M.; Burke, A.J. Petasis adducts of tryptanthrin – synthesis, biological activity evaluation and druglikeness assessment. *New J. Chem.* **2021**, *45*, 14633–14649, https://doi.org/10.1039/d1nj02079j.

5.14. Graphene Oxide-Assisted Delivery of Cationic Zn(II) Phthalocyanines to DNA G-Quadruplexes

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The stabilization of G-quadruplex (G-Q) in deoxyribonucleic acid (DNA) telomeres by phthalocyanines (Pcs) has been described as a potential anticancer strategy, since it inhibits the activity of telomerase [1]. However, the typical aggregation of Pcs in aqueous systems strongly compromises their potential as G-Q ligands. Herein, we evaluated the potential of a tetracationic Zn(II) thiopyridinium phtalocyanine (ZnPcA) and three structurally related octacationic ZnPcs (ZnPcB-D) as G-Q ligands after their immobilization on graphene oxide (GO) nanosheets.

The re-organization of the ZnPcs occurred from H-aggregates to J-aggregates, as suggested by optical measurements performed on ZnPc@GO hybrid materials. Attending to the clear-cut differences in the fluorescence emission intensities of ZnPcs (fluorescent) and ZnPc@GO hybrids (quenched), we decided to carry out spectrofluorimetric titrations of each ligand with DNA structures (duplex ds26 and G-Q (T2G5T)4). The fluorescence of ZnPcA@GO and ZnPcB@GO was recovered and enhanced after the titrations with DNA structures, with both hybrid materials showing similar affinity to G-Q structures. On the other hand, ZnPcC and ZnPcD showed undesirable aggregation in the tested conditions.

The monitoring of the fluorescence emission over 1 week suggested a progressive release of ZnPcA and ZnPcB from ZnPc@GO hybrids to form stable ZnPc/DNA complexes. We suggest that GO acts as a nanoplatform that temporarily immobilizes ZnPcs and allows the gradual and selective release of ZnPcs to stabilize G-Q along time, similarly to other porphyrin@GO systems [2]. These systems can also be regarded as "turn on–off–on" fluorescence sensors, which are valuable for developing theragnostic G-Q sensors (Figure 1).

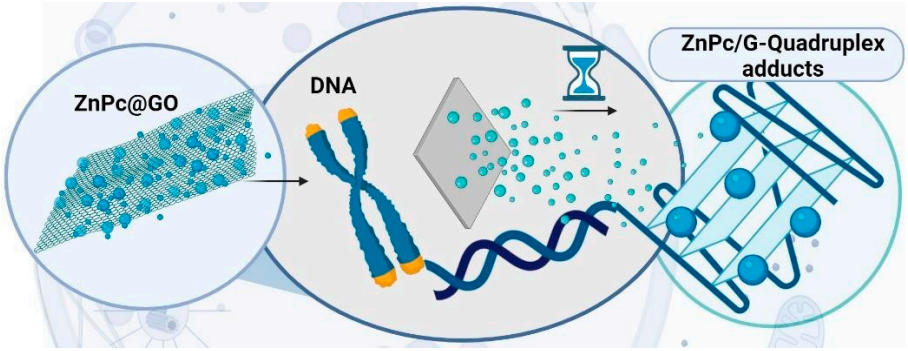

Figure 1. Stabilization of G-Q by the release of ZnPcs from GO nanosheets.

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References

1. Ramos, C.; Almeida, S.; Lourenço, L.; Pereira, P.; Fernandes, R.; Faustino, M.; Tomé, J.; Carvalho, J.; Cruz, C.; Neves, M. Multicharged Phthalocyanines as Selective Ligands for G-Quadruplex DNA Structures. *Molecules* **2019**, *24*, 733, https://doi.org/10.3390/molecules24040733.
2. Monteiro, A.R.; Ramos, C.I.V.; Fateixa, S.; Moura, N.M.M.; Neves, M.G.P.M.S.; Trindade, T. Hybrids Based on Graphene Oxide and Porphyrin as Tools for Detec-

tion and Stabilization of DNA G-Quadruplexes. *ACS Omega* **2018**, *3*, 11184–11191, https://doi.org/10.1021/acsomega.8b01366.

5.15. Chromeno[3,4-b]xanthones: First-in-Class AChE and Aβ Aggregation Dual Inhibitors

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The multifactorial nature of Alzheimer's disease (AD) has been claimed as one of the main reasons for the current lack of effective drug therapies. This health problem is quite understandable if one thinks about the complex biology of associated to AD, which is difficult to reduce to a single target whose modulation will impact the broad spectrum of pathologies and symptoms [1,2]. This is on the basis of an alternative paradigm—the multitarget directed ligands (MTDLs)—with the final goal of modulating more than one drug target resorting to one single molecular entity.

Herein, the framework combination approach (the gold standard for new MTDLs) was explored to prepare chromeno[3,4-*b*]xanthones, which are meant to act as AChE and Aβ aggregation dual inhibitors [1,2]. The preliminary structure–activity relationship (SAR) profile showed that chromeno[3,4-*b*]xanthones are capable of inhibiting AChE in low micromolar range (IC_{50} = 2.1–6.9 μM) (Figure 1) [3]. The introduction of an OMe group in the chromeno[3,4-*b*]xanthone scaffold increased their potency against Aβ aggregation up to 70% with only a small loss in AChE inhibition (IC_{50} = 3.9 μM) (Figure 1) [3]. The synthetic strategy as well as the detailed SAR profile of these compounds will be presented and discussed under the concept of MTDLs.

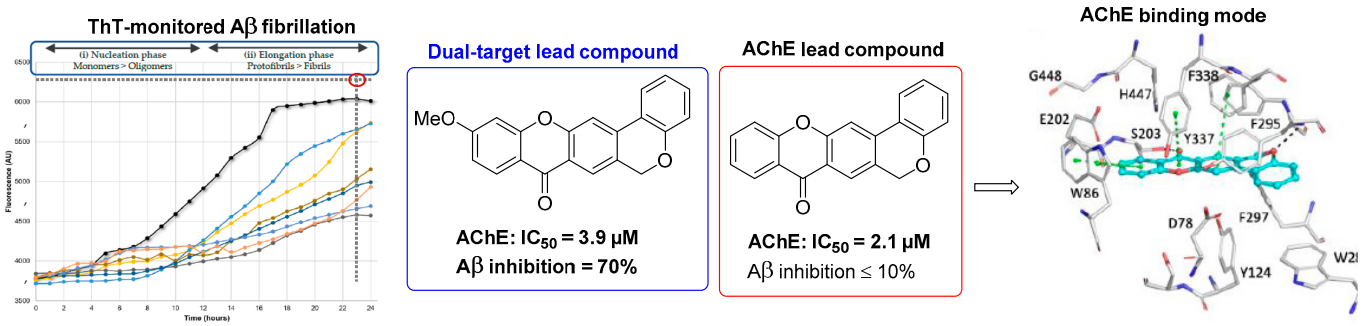

Figure 1. Chromeno[3,4-*b*]xanthones as first-in-class AChE and Aβ aggregation inhibitors.

Funding: This work was financed by Portugal 2020 through FEDER in the frame of POCI and in the scope of the projects: PAGE project "Protein aggregation across the lifespan" (CENTRO-01-0145-FEDER-000003), and LAQV-REQUIMTE (UIDB/50006/2020), which are co-financed by FCT/MCTES. This work was financed by National Funds through FCT/MCTES—Portuguese Foundation for Science and Technology within the scope of the project UIDB/50006/2020. H.M.T.A. acknowledges EpigenGlicON project (POCI-01-0145-FEDER-029767) for his researcher contract.

References

1. Benek, O.; Korabecny, J.; Soukup, O. A Perspective on Multi-target Drugs for Alzheimer's Disease. *Trends in Pharmacol. Sci.* **2020**, *41*, 434.

2. Malafaia, D.; Albuquerque, H.M.T.; Silva, A.M.S. Amyloid-β and tau aggregation dual-inhibitors: A synthetic and structure-activity relationship focused review. *Eur. J. Med. Chem.* **2021**, *214*, 113209.
3. Malafaia, D.; Oliveira, A.; Fernandes, P.A.; Ramos, M.J.; Albuquerque, H.M.T.; Silva, A.M.S. Chromeno[3,4-b]xanthones as First-in-Class AchE and Aβ Aggregation Dual-Inhibitors. *Int. J. Mol. Sci.* **2021**, *22*, 4145.

5.16. New Acenaphthylene-Based Sensitizers for DSSC Applications

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Dyes containing an acenaphthylene core have not received yet much interest to be applied in DSSCs when compared with the phenanthrene-based dyes [1]. A new set of novel compounds containing an acenaphthylene backbone was synthesized: namely, derivatives of 1,2-diethynylacenaphthylene containing phenyl, thiophene, benzotriazole and thieno-[3,2-b]thiophene rings present in their π-bridge. The synthesis of these compounds requires Sonogashira coupling reactions to introduce the ethynyl moiety and the aromatic rings. Photophysical and spectroscopic studies were performed to predict their applicability in DSSCs (Figure 1).

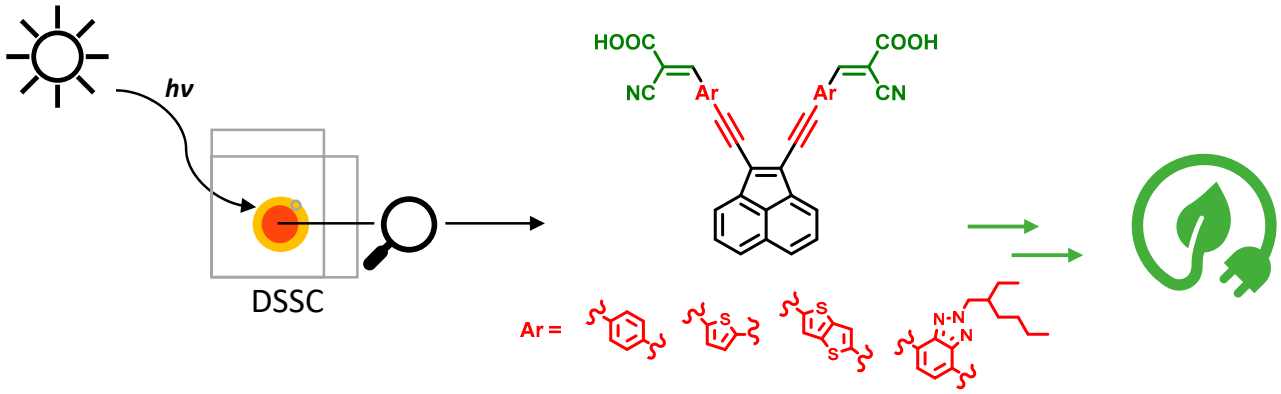

Figure 1. Schematic representation of the synthesized acenaphthylene-based organic dyes.

Funding: This work was performed under the projects PTDC/QUI-QOR/7450/2020 "Organic Redox Mediators for Energy Conversion" through the FCT—Fundação para a Cincia e a Tecnologia I. P. This work was also supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020). FCT/MCTES is also acknowledged for the National NMR Facility Network (ROTEIRO/0031/2013-PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI, PORL, and FCT through PIDDAC) and PhD grant PD/BD/145324/2019/(G.M.).

Reference

1. Sivanadanam, J.; Aidhen, I.S.; Ramanujam, K. New cyclic and acyclic imidazole-based sensitizers for achieving highly efficient photoanodes for dye-sensitized solar cells by a potential-assisted method, *New J. Chem.* **2020**, *44*, 10207–10219.

5.17. One-Pot Functionalization of a 7-Hydroxy Amino-Based Pyranoflavylium Compound: The Power of Coupling Chemistry to Synthetize New Esters

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Over the years, the chemical modification of anthocyanins and/or derivatives remains a constant challenge. Electronic conjugation and delocalization properties around the oxonium moiety and the occurrence of dynamic equilibrium forms in solution affect the reactivity and the availability of these compounds [1]. Previous works reported the chemical and enzymatic (using Candida antarctica lipase B) acylation of quercetin-3-O-glucoside [2], malvidin-3-O-glucoside (Mv3glc) and extracts [3]. The results showed a significant increase in the thermal stability compared to the precursor. However, these esterifications were achieved at the primary C6''–OH of Mv3glc with a conversion yield around 20%. This work proposes a new approach for the functionalization of a 7-hydroxy amino-based pyranoflavylium compound using one-step 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) coupling chemistry (Figure 1). Different cinnamic acids were studied to establish an ester bond with the 7-hydroxy group of the pyranoflavylium compound, including a *trans*-cinnamic acid, 4-(dimethylamino), 4-bromo and 4-amino cinnamic acids. The carboxylic group present in their structure is activated by the coupling agent (EDC) and then, it reacts with the hydroxyl group at carbon C-7 of ring A of the cation pyranoflavylium structure. This work presents for the first-time the chemical modification of aromatic hydroxyl groups of a pyranoflavylium compound. The high reaction yields and the improvement of physicochemical properties with a bathochromic shift (red region) on the maximum absorption wavelength, indicating a higher stability toward a wide pH range compared with the precursor pyranoflavylium, make these new functionalized compounds promising for biomedical applications including photodynamic therapy.

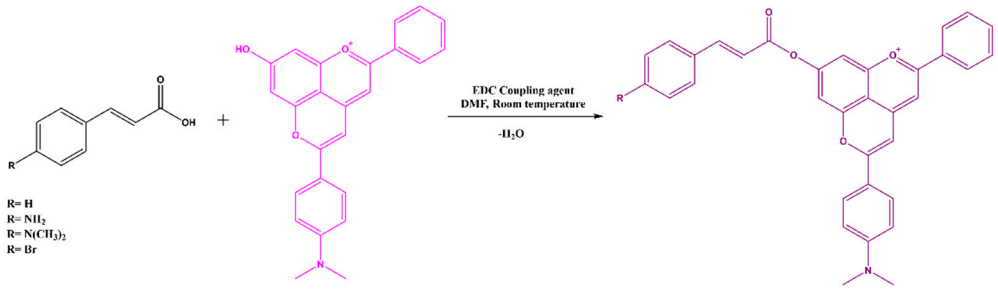

Figure 1. Functionalization of a 7-hydroxy amino-based pyranoflavylium compound using one-pot 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) coupling chemistry.

Funding: We thank the FCT for a doctoral grant from (SFRH/BD/146549/2019) and the Associated Laboratory for Sustainable Chemistry, Clean Processes and Technologies LAQV-REQUIMTE through the national funds from UIDB/50006/2020 and UIDP/50006/2020. The authors also thank AgriFood XXI I&D&I project (NORTE-01-0145-FEDER-000041) co-financed by the European Regional Development Fund (ERDF) through the NORTE 2020 (Programa Operacional Regional do Norte 2014/2020).

References

1. Dangles, O.; Fenger, J.-A. The Chemical Reactivity of Anthocyanins and Its Consequences in Food Science and Nutrition. *Molecules* **2018**, *23*, 1970, https://doi.org/10.3390/molecules23081970.

2. Ishihara, K.; Nakajima, N. Structural aspects of acylated plant pigments: stabilization of flavonoid glucosides and interpretation of their functions. *J. Mol. Catal. B Enzym.* **2003**, *23*, 411–417.
3. Cruz, L.; Fernandes, V.; Araújo, P.; Mateus, N.; Freitas, V. Synthesis, characterisation and antioxidant features of procyanidin B4 and malvidin-3-glucoside stearic acid derivatives. *Food Chem.* **2015**, *174*, 480–486, https://doi.org/10.1016/j.foodchem.2014.11.062.

5.18. Diazaborines as Stable and ROS-Responsive Linkers: The Dawn of a New Generation of Antibody–Drug Conjugates

João P. M. António [1], Joana Inês Carvalho [1], Ana S. André [2], Joana N. R. Dias [2], Sandra I. Aguiar [2], Hélio Faustino [1], Ricardo M. R. M. Lopes [1], Luis F. Veiros [3], Gonçalo J. L. Bernardes [4,5], Frederico A. da Silva [2] and Pedro M. P. Gois [1]

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Antibody–drug conjugates (ADCs) are one of the most promising classes of therapeutics in the battle against cancer. By merging the exceptional targeting ability of antibodies and the potency of powerful cytotoxic drugs, ADCs display high levels of selectivity, tolerability and cytotoxic activity. The success of an ADC is closely related with the careful optimization of its four major components: antibody, payload, linker and bioconjugation technology. The linker, in particular, must be stable in solution and capable of releasing the payload upon a predetermined stimulus [1]. Current ADCs explore the distinctive microenvironment of cancer cells to ensure a selective deliver of the drug, including its acidic pH, high glutathione levels and overexpressed proteolytic enzymes (Figure 1).

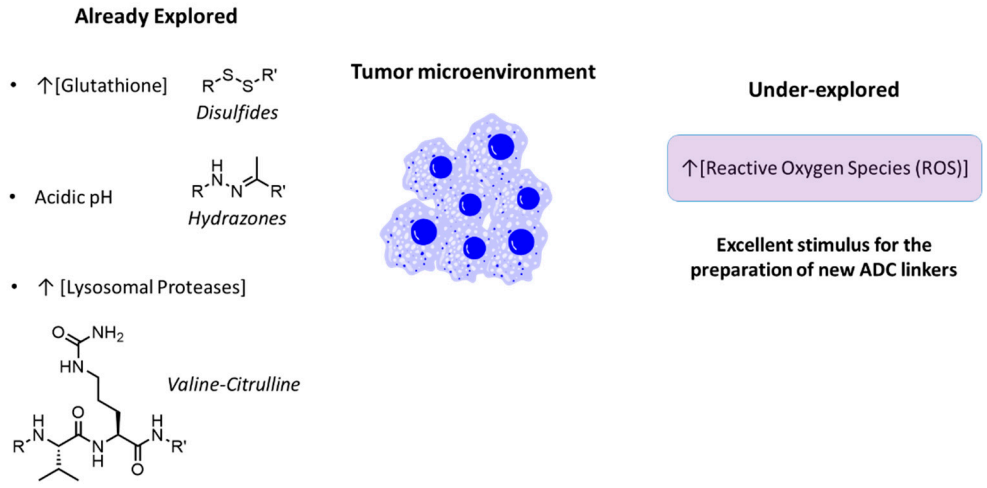
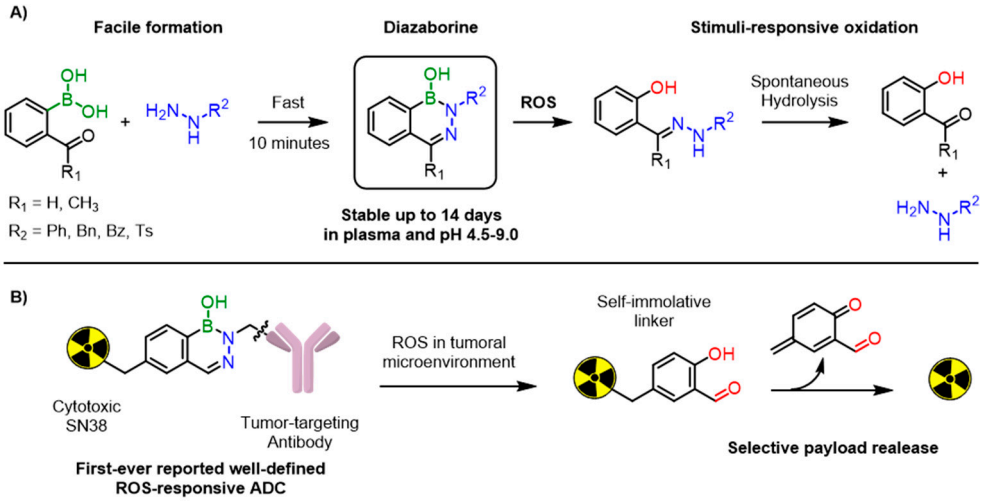


Figure 1. Contrary to glutathione, acidic pH and proteases, the increased concentration of ROS in the tumor microenvironment has not been explored as a stimuli for the preparation of ADCs.

In this work, we demonstrate for the first time that the high reactive oxygen species (ROS) concentrations present in tumor cells can be exploited to generate a first-in-class

ROS-responsive ADC [2]. The synthesis of this ADC was possible due to the discovery that diazaborines (DABs) are a very effective ROS-responsive unit while being stable in buffer and in plasma. DABs can be generated with click-like kinetics (bioorthogonal, 10 min aqueous pH 7.4) and displayed remarkable stability in pH 4.5–9.0 and plasma. However, in the presence of 100 equiv. H_2O_2, they were swiftly oxidized ($t_{1/2}$ = 15 min) (Scheme 1A). Mechanistic and DFT experiments were performed on the system to further understand the details behind their stability and selectivity.



Scheme 1. (**A**) Diazaborines have fast formation kinetics, high stability in buffer and plasma, and are oxidized swiftly in the presence of ROS; (**B**) We designed a diazaborine-based ADC featuring the cytotoxic drug SN-38.

To showcase their potential, a DAB-based self-immolative linker was designed and used in the construction of a homogenous ADC. The ADC, featuring a SN-38 cytotoxic drug and a B-cell lymphoma targeting antibody, showed remarkable activity (IC_{50} = 54.1 nM) and selectivity (>100 μM in T-cell lymphoma) (Scheme 1B). Due to their modularity and fast kinetics, we envision that DABs will play an important role in the development of a new generation of ROS-responsive linkers which could span from the construction of additional ADCs to the development of novel responsive materials.

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References

1. António, J.P.M.; Russo, R.; Carvalho, C.P.; Cal, P.M.S.D.; Gois, P.M.P. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates *Chem. Soc. Rev.* **2019**, *48*, 3513.
2. António, J.P.M.; Carvalho, J.I.; André, A.S.; Dias, J.N.R.; Aguiar, S.I.; Faustino, H.; Lopes, R.M.R.M.; Veiros, L.F.; Bernardes, G.J.L.; Silva, F.A.; Gois, P.M.P. Diazaborines are a Versatile Platform to Develop ROS-Responsive Antibody Drug Conjugates. *Angew. Chem. Int. Ed.* **2021**, *60*, 25914.

5.19. Application of Immobilized epi-Cinchona Alkaloids in Organocatalysis

Ana C. Amorim [1,2,*], Daniela P. Fonseca [1,2], Elisabete Carreiro [3], Gesine J. Hermann [1,2], Hans-Jürgen Federsel [1,2], Ana Rita C. Duarte [4], Daria Brooks [5], Matthew Thompson [5] and Anthony J. Burkeb [3,6,*]

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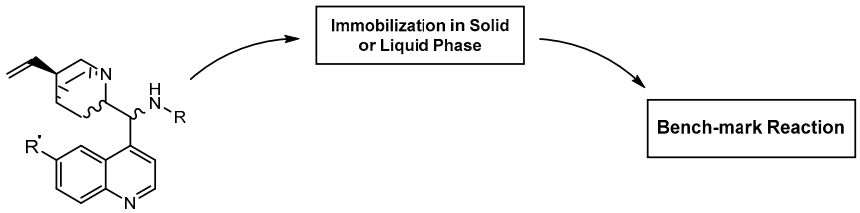
Organocatalysis has now been clearly established as the third pillar of catalysis after metal-based catalysis and biocatalysis with the award of the Nobel prize in chemistry to List and MacMillan in 2021. For two decades, cinchona alkaloids have been used with great success as organocatalysts in the field of asymmetric organocatalysis [1–3]. They have been explored in many types of chemical transformation, which include aldol condensations, Michael additions, Henry reactions, Mannich reactions, etc. The disadvantage is that the catalytic loadings are generally in the order of 20 mol %; thus, from an economic and environmental point of view, it makes sense to recover and recycle the catalyst. This can be achieved via immobilization of the catalyst to a solid or in an appropriate liquid. In this study, we tested a group of immobilized epi-cinchona alkaloids in some well-known benchmark reactions, evaluating the yield of the reaction, the enantioselectivity and the number of cycles where the catalyst remains reactive (Scheme 1). For the immobilization, we have used both betaine-based deep eutetic solvents (DESs) and specific Controlled Porous Glass Beads (CPGs)—i.e., EziG Opal, EziG Coral and EziG Amber—supplied from EnginZyme (www.enginzyme.com, accessed on 19 August 2022) [4].



Scheme 1. Strategy used to prepare and test our immobilized organocatalysts.

Both methods gave good yields and high enantioselectivities; a reasonable number of catalytic cycles could be achieved, as discussed in this presentation.

Funding: We thank the FCT for funding to LAQV-REQUIMTE through project UIDB/50006/2020.

References

1. Burke, A.J.; Hermann, G.J. 3 Amino-cinchona derivatives. In *Organocatalysis: Stereoselective Reactions and Applications in Organic Synthesis*, 1st ed.; Benaglia, M., Eds; De Gruyter: Berlin, Germany, 2021; pp. 85–176, https://doi.org/10.1515/9783110590050-003.

2. Parvez, M.M.; Haraguchi, N.; Itsuno, S. Synthesis of Cinchona Alkaloid-Derived Chiral Polymers by Mizoroki–Heck Polymerization and Their Application to Asymmetric Catalysis. *Macromolecules* **2014**, *47*, 1922–1928, https://doi.org/10.1021/ma5001018.
3. Singh, G.; Yeboah, E. Recent applications of Cinchona alkaloid-based catalysts in asymmetric addition reactions. *Rep. Org. Chem.* **2016**, *6*, 47–75.
4. Cassimjee, K.; Kadow, M.; Wikmark, Y.; Humble, M.; Rothstein, M.; Rothstein, D.; Bäckvall, J. A general protein purification and immobilization method on controlled porosity glass: biocatalytic applications. *Chem. Commun.* **2014**, *50*, 9134.

5.20. The DMPU as Green Solvent in Flavonoid Syntheses

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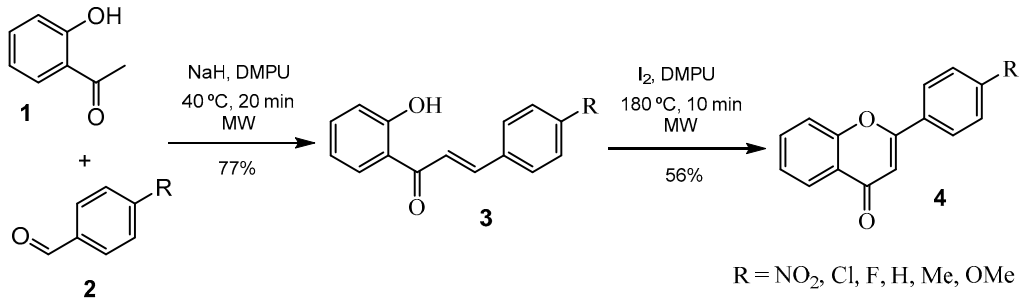
Humanity faces troubled times with the pandemic and climate change. Researchers are incumbent to address these issues, namely finding more sustainable ways to live our lives. The high organic solvent volumes used in the chemical industry, many of them harmful to the environment, impose a significant constraint toward our common goal [1].

Although eradicating the solvents seems impossible for now, substituting them for greener options is a must. The compound 1,3-dimethyl-1,3-diazinan-2-one (*N*,*N*′-dimethylpropyleneurea, DMPU) can be used as a green dipolar aprotic solvent with excellent properties for organic reactions. It has been tested for a condensation reaction in the chalcone 3 synthesis (taking advantage of its high hygroscopicity) and then for the chalcone oxidation to flavone 4 under microwave radiation (Scheme 1). Its characterization has also been completed with analysis of solvatochromic parameters, densities, viscosities, heat capacities and ^{1}H, and ^{13}C NMR spectra of mixtures with water [2,3].



Scheme 1. Chalcone and flavone syntheses in DMPU under microwave irradiation.

The reactions' yields were generally good (up to 77%). The main advantages of this solvent were the very low microwave potencies required (around 10 W), good compounds' solubilities, low vapor pressures, and versatility in the purification process (products' precipitation with water or dichloromethane addition). The characterization studies proved the formation of strong interactions with water molecules, with a sharp increase in density and viscosity for mixtures with water of composition xDMPU ≈0.35 [2,3].

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References

1. Anastas, P.; Eghbali, N. Green Chemistry: Principles and Practice. *Chem. Soc. Rev.* **2010**, *39*, 301–312.
2. Santos, R.A.L.S. A DMPU's study: characterization and applicability as solvent in organic reactions. Master's Thesis, University of Aveiro, Aveiro, Portugal, 2020.
3. Santos, R.A.L.S.; Magalhães, M.C.F.; Hefter, G.; Akilan, C.; Martins, M.A.R.; Carvalho, P.J.; Pinto, D.C.G.A. Densities, heat capacities, viscosities, 1H- and 13C-NMR spectra, and solvatochromic parameters of binary mixtures of 1,3-dimethyl-1,3-diazinan-2-one (DMPU) and water. *J. Chem. Thermodyn.* **2021**, *161*, 106550.

5.21. An Attractive Ligand for the Development of G4 Labeling Probes

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Telomerase and oncogene promotors are closely associated with tumor occurrence; thus, these structures are being recognized as targets for the development of new anticancer drugs [1,2]. Telomerase is expressed in a range of cancer cells, and stabilization of the G-quadruplexes (G4) structures in the terminal region of the telomeres has been reported to inhibit telomerase activity. G4s are DNA secondary structures reported to be found in several genome regions of biological significance, especially at the ends of the chromosomes, the telomeres [1,2]. Telomeres act as chromosome "sealants" stabilizing the linear strands and preventing their damage. In normal somatic cells, telomeres are shortened in the process of DNA replication and eventually become too short to protect the chromosome, leading to cell senescence and death. Many cancer cells can counteract this shortening by increasing the level of activity of telomerase, which is a reverse transcriptase enzyme that allows continuous cell division without telomere shortening.

The efficacy of several molecules in telomerase inhibition and regulation of genes expression, by adduct formation with G-quadruplexes (Figure 1), has been studied by biophysical and biochemical methods with promising results [3–6]. We report here the synthesis and structural characterization of a small positively charged ligand that showed very promising results as a G4-stabilizing ligand, being particularly selective for oncogene promotors [7].

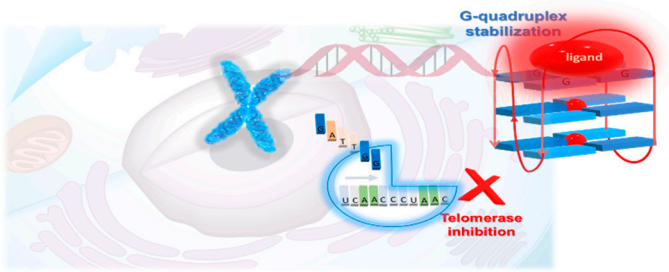

Figure 1. Indirect telomerase inhibition by G-quadruplex stabilization by ligands.

Data obtained from different spectroscopic and in vitro experiments showing that the studied molecule presents high affinity to G4 structures will be discussed. Docking studies

and molecular dynamics simulations unravelling the binding modes of the ligand with different G4 will also be presented.

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References

1. Collie, G.W.; Parkinson G.N. The application of DNA and RNA G-quadruplexes to therapeutic medicines. *Chem. Soc. Rev*. **2011**, *40*, 5867–5892, https://doi.org/10.1039/C1CS15067G.
2. Nandakumar, J.; Cech, T.R. Finding the end: recruitment of telomerase to telomeres. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 69–82, https://doi.org/10.1038/nrm3505.
3. Monteiro A.R.; Ramos C.I.V.; Fateixa S.; Moura N.M.M.; Neves M.G.P.M.S.; Trindade, T. Hybrids Based on Graphene Oxide and Porphyrin as Tools for Detection and Stabilization of DNA G-Quadruplexes. *ACS Omega* **2018**, *3*, 11184–11191, https://doi.org/10.1021/acsomega.8b01366.
4. Ramos, C.I.V.; Almeida, S.P.; Lourenço, L.M.O.; Pereira, P.M.R.; Fernandes, R.; Faustino, M.A.F.; Tomé, J.P.C.; Carvalho, J.; Cruz, C.; Neves, M.G.P.M.S. Multicharged Phthalocyanines as Selective Ligands for G-Quadruplex DNA Structures. *Molecules* **2019**, *24*, 733, https://doi.org/10.3390/molecules24040733.
5. Lopes-Nunes, J.; Carvalho, J.; Figueiredo, J.; Ramos, C.I.V.; Lourenço, L.M.O.; Tomé, J.P.C.; Neves, M.G.P.M.S.; Mergny, J.-L.; Queiroz, J.A.; Salgado, G.F.; Cruz C. Phthalocyanines for G-quadruplex aptamers binding. *Bioorg. Chem.* **2020**, *100*, 103920, https://doi.org/10.1016/j.bioorg.2020.103920
6. Ramos, C.I.V.; Monteiro, A.R.; Moura, N.M.M.; Faustino, M.A.F.; Trindade, T.; Neves, M.G.P.M.S. The Interactions of H2TMPyP, Analogues and Its Metal Complexes with DNA G-Quadruplexes—An Overview. *Biomolecules* **2021**, *11*, 1404.
7. Ramos, C.I.V.; Almodôvar, V.A.S.; Candeias, N.; Santos, T.; Cruz, C.; Neves, M.G.P.M.S.; Tomé, A.C. Diketopyrrolo[3,4–c]pyrrole derivative as a promising ligand for the stabilization of G-quadruplex DNA structures. *Bioorg. Chem.* **2022**, *122*, 105703, https://doi.org/10.1016/j.bioorg.2022.105703.

5.22. Photoactivated Cell-Killing Amino-Based Flavylium Compounds

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Six amino-based flavylium dyes (5OH4′NMe_2, 5,7$OH_2$4′NMe_2, 5,7OH_2st4′NMe_2, 7$NEt_2$4′NMe_2, 7NEt_2st4′NMe_2 and 7$NEt_2$4′NH_2) were synthesized through an acidic aldolic condensation reaction between 2,4,6-trihydroxybenzaldehyde or 4-diethylaminosalysaldehyde with different acetophenones (4-amino- or 4-dimethylamino–acetophenone or *p*-(dimethylamino)-phenyl-but-3-en-2-one). Compounds presenting a 2-styryl linkage have a higher maximum absorption wavelength, which is probably resulting from their extended electronic delocalization. On the other hand, the presence of stronger electron-donating groups such as dimethyl- or diethyl-amino leads to a bathochromic shift in the maximum absorption wavelength of dyes when compared to the ones presenting hydroxyl groups or a primary amine [1].

This work also reports the study of their potential as photosensitizers toward topical PDT. 7NEt$_2$4′NMe$_2$, 7NEt$_2$st4′NMe$_2$ and 7NEt$_2$4′NH$_2$ showed significant fluorescence quantum yields (from 3.40 to 20.20%) and production of singlet oxygen ($^1O_2$). IC50 values regarding the growth inhibition of keratinocytes were between 0.9 and 1.5 μM after 10 min of photoactivation with a white light. This cellular damage in keratinocyte cells upon white light activation was accompanied with the production of reactive oxygen species (ROS). It was also found that the compounds can induce damage by either a type I (ROS production) or type II (singlet oxygen) PDT mechanism, although a higher cell survival was observed in the presence of $^1O_2$ quenchers. Overall, a structure–activity relationship could be established, ranking the most important functional groups for the photoactivation efficiency as follows: C7-diethylamino > C4′-dimethylamino > C2-styryl [2].

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References

1. Araújo, P.; Rita Pereira, A.; de Freitas, V.; Mateus, N.; Fernandes, I.; Oliveira, J. Synthesis, structural characterization and chromatic features of new 2-phenyl-1-benzopyrylium and 2-phenyl-styryl-1-benzopyrylium amino-based blue dyes. *Tetrahedron Lett.* **2021**, *85*, 153487, https://doi.org/10.1016/j.tetlet.2021.153487.
2. Oliveira, H.; Araújo, P.; Pereira, A.R.; Mateus, N.; de Freitas, V.; Oliveira, J.; Fernandes, I. Photoactivated cell-killing amino-based flavylium compounds. *Sci. Rep.* **2021**, *11*, 22005, https://doi.org/10.1038/s41598-021-01485-y.

5.23. Quercetin Liposomes: An Effective Anti-Inflammatory Treatment for Hepatic Ischemia and Reperfusion Injury Demonstrated In Vitro and In Vivo

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Quercetin is a flavonoid with anti-inflammatory and antioxidant properties [1]. However, its fast liver metabolism and poor bioavailability result in a low in vivo therapeutic efficacy [2]. To overcome these drawbacks, quercetin was incorporated in long-circulating liposomes that were evaluated for the treatment of hepatic ischemia and reperfusion injury (IRI) (Figure 1).

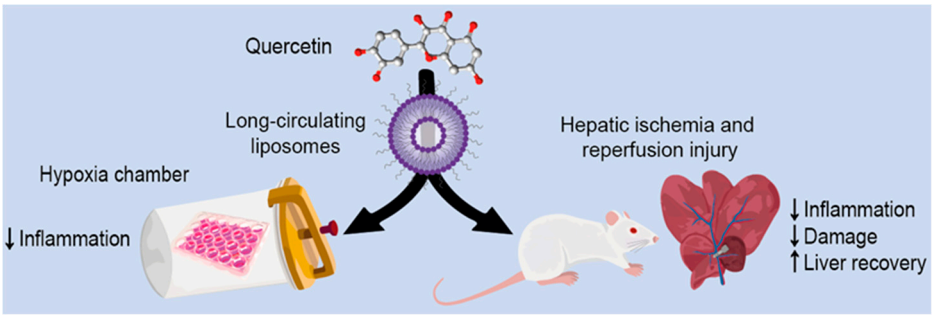

Figure 1. In vitro and in vivo evaluation of the anti-inflammatory potential of quercetin liposomes.

Stable quercetin liposomes (Q-Lip) were developed and characterized, presenting a mean diameter under 0.13 μm with a low polydispersity index, zeta potential values around zero (mV) at pH 6.0 and a quercetin to lipid ratio of 31 ± 3 μg/μmol. An in vitro model of hypoxia was optimized, and the treatment with Q-Lip resulted in the reduction in pro-inflammatory cytokines expression, demonstrating the anti-inflammatory potential of the liposomal formulation in comparison to free quercetin. This potential was confirmed in an in vivo rodent model of hepatic IRI, where the intravenous injection of Q-Lip 24 h before the surgical procedure resulted in a reduction in serum transaminase levels, a decrease in TNF-α mRNA expression and an improvement of liver recovery after the surgery. Altogether, these results demonstrate that liposomes greatly improve the therapeutic potential of quercetin and that Q-Lip are a promising alternative for hepatic IRI treatment.

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References

1. Proença, C.; Ribeiro, D.; Soares, T.; Tomé, S.M.; Silva, A.M.S.; Lima, J.L.F. C.; Fernandes, E.; Freitas, M. Chlorinated Flavonoids Modulate the Inflammatory Process in Human Blood. *Inflammation* **2017**, *40*, 1155–1165.
2. D'Andrea, G. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia* **2015**, *106*, 256–271.

5.24. Synthesis and Structural Characterization of a New Brominated Pyranoflavylium Dye for Application in Photodynamic Therapy

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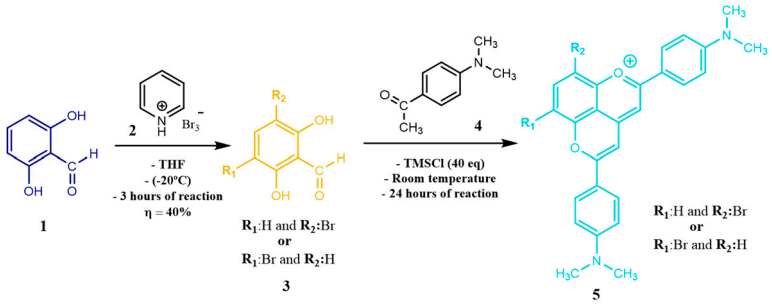
Photodynamic therapy (PDT) is a well-established therapeutic for the treatment of different inflammatory skin diseases such as acne vulgaris, actinic keratosis, psoriasis, sarcoidosis, and several infectious skin diseases and non-melanoma skin cancers. PDT uses non-toxic dyes as photosensitizers (PSs), which are activated by the absorption of visible light to initially form the excited singlet state, followed by transition to the long-lived excited triplet state. This triplet state can undergo photochemical reactions in the presence of oxygen to form reactive oxygen species (including singlet oxygen) that can destroy cancer cells, pathogenic microbes and unwanted tissues [1].

Tetrapyrrole structures have been widely investigated in PDT. The addition of heavy halogen atoms (as Br, I, F and Cl) in the pyrrole rings increases the triplet yield and allows the molecules to function as PSs [2]. Bearing this, the present work reports the synthesis (Scheme 1) of a new brominated pyranoflavylium dye (5) as a photosensitizer toward topical PDT, which results from the acidic aldolic condensation reaction between a 3-bromo-2,6-dihydroxybenzaldehyde or 5-bromo-2,6-dihydroxybenzaldehyde (3) and an excess of 4-dimethylamino-acetophenone (4). The 3-bromo-2,6-dihydroxybenzaldehyde or 5-bromo-2,6-dihydroxybenzaldehyde (3) were obtained from the reaction between 2,6-dihydroxybenzaldehyde (1) and pyridium tribromide (2) in tetrahydrofuran.



Scheme 1. Synthetic route of the new brominated pyranoflavylium.

The structure characterization of this new dye (5) was also performed by LC-MS and NMR (1H, 13C, HMBC, HSQC and gCOSY) analysis. The pigment exhibits an intense turquoise blue color with a maximum absorption wavelength in the red region.

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References

1. Abrahamse, H.; Hamblin, M.R. New photosensitizers for photodynamic therapy. *Biochem. J.* **2016**, *473*, 347–364, https://doi.org/10.1042/bj20150942.
2. Kamkaew, A.; Lim, S.H.; Lee, H.B.; Kiew, L.V.; Chung, L.Y.; Burgess, K. BODIPY dyes in photodynamic therapy. *Chem. Soc. Rev.* **2012**, *42*, 77–88, https://doi.org/10.1039/c2cs35216h.

5.25. Lead Optimization of Azaaurones as Novel Chemotypes against Mycobacterium Tuberculosis

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Tuberculosis (TB), caused by the bacillus Mycobacterium tuberculosis (M.tb), is the second leading cause of death from a single infectious agent worldwide after COVID-

19. The complexity and duration of the treatment lead to misuse and low compliance by patients, increasing disease burden and the appearance of multidrug-resistant strains. New antibiotics active against drug-resistant M.tb with shorter therapeutic regimens are urgently needed [1,2]. A family of azaaurone-based derivatives, from a chemical library developed in iMed.ULisboa, was revealed to be active against M.tb, including multidrug- and extensively drug-resistant tuberculosis from clinical isolates, at a submicromolar level [3]. Despite the promising activities, this new scaffold displayed poor ADME properties. We now report the complete SAR exploration and ADME profiling of newly synthesized derivatives. Along with an enhanced metabolic stability and solubility, rings A and B as well as N-substitutions were extensively explored. (Figure 1) Reduction in the exocyclic carbon–carbon double bond was also performed, generating a new family of saturated azaaurone analogs devoid of a Michael acceptor moiety.

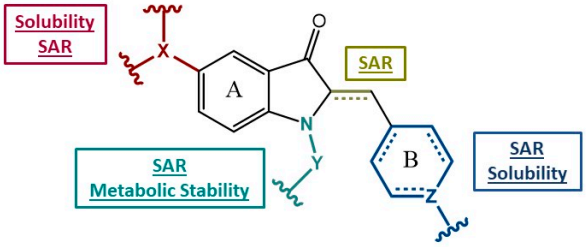

Figure 1. Derivatization of the azaaurone scaffold, with main objectives per moiety.

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References

1. Campaniço, A.; Moreira, R.; Lopes, F. Drug discovery in tuberculosis. New drug targets andantimycobacterial agentes. *Eur. J. Med. Chem.* **2018**, *150*, 525–545.
2. Ehrt, S.; Schnappinger, D. Mycobacterial survival strategies in the phagosome: defence against host stresses. *Cell. Microbiol.* **2009**, *11*, 1170–1178.
3. Campaniço, A.; Carrasco, M.P.; Njoroge, M.; Seldon, R.; Chibale, K.; Perdigão, J.; Portugal, I.; Warner, D.F.; Moreira, R.; Lopes, F. Azaaurones as Potent Antimycobacterial Agents Active against MDR- and XDR-TB. *ChemMedChem* **2019**, *14*, 1537–1546.

5.26. Searching for Antiaging Compounds—Strategies and Challenges

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Aging is a multifactorial process that is caused by both intrinsic and extrinsic factors, and it is generally noticed at first by changes in skin structure such as wrinkles, sagging

and pigmentation spots. Skin aging is mainly due to the deleterious effect of oxidant species, exposure to UV radiation, and to dramatic changes in the extracellular matrix (ECM) structure due to an imbalance between the synthesis and degradation of collagen, elastin and hyaluronic acid [1,2]. Skin antiaging strategies and antiaging compounds are an important field of research, since maintaining the structure of skin contributes not only to a higher self-esteem but also to preserve an organ that is our main barrier against external aggressions. Among antiaging strategies, there are those that seek to stimulate fibroblast and keratinocyte viability, and there are those that act by minimizing degradative processes due to oxidant species and to the increased activity of enzymes that hydrolyze ECM components [3].

Natural products from terrestrial plants and marine algae are excellent sources of compounds that counteract oxidative processes and inhibit the activity of collagenase, elastase, hyaluronidase and tyrosinase [4,5].

We have been searching for antiaging natural products, obtained from Azorean and Macaronesia species, as well as compounds derived from natural products scaffolds. The most interesting results related with bioactive extracts and pure compounds and the chemical structure of the latter, some of them already published, will be presented and discussed.

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References

1. Haydont, V.; Bernard, B.A.; Haydont, N.O.F. Age-related evolutions of the dermis: Clinical signs, fibroblast and extracellular matrix dynamics. *Mech. Ageing Dev.* **2019**, *177*, 150–156.
2. Shin, J.-W.; Kwon, S.-H.; Choi, J.-Y.; Na, J.-I.; Huh, C.-H.; Choi, H.-R.; Park, K.-C. Molecular Mechanisms of Dermal Aging and Antiaging Approaches. *Int. J. Mol. Sci.* **2019**, *20*, 2126.
3. Espinosa-Leal, C.A.; Garcia-Lara, S. Current Methods for the Discovery of New Active Ingredients from Natural Products for Cosmeceutical Applications. *Planta Medica* **2019**, *85*, 535–551, https://doi.org/10.1055/a-0857-6633.
4. Ding, A.-J.; Zheng, S.; Huang, X.-B.; Xing, T.-K.; Wu, G.-S.; Sun, H.-Y.; Qi, S.-H.; Luo, H.-R. Current Perspective in the Discovery of Anti-aging Agents from Natural Products. *Nat. Prod. Bioprospecting* **2017**, *7*, 335–404, https://doi.org/10.1007/s13659-017-0135-9.
5. Zárate, R.; Portillo, E.; Teixidó, S.; Carvalho, M.; Nunes, N.; Ferraz, S.; Seca, A.; Rosa, G.; Barreto, M. Pharmacological and Cosmeceutical Potential of Seaweed Beach-Casts of Macaronesia. *Appl. Sci.* **2020**, *10*, 5831, https://doi.org/10.3390/app10175831.

5.27. Reverting the Membrane-Interfering Behavior of Polyphenols via C-Glucosylation: A New Molecular Design Tool in Drug Discovery

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Pan-Assay Interference CompoundS (PAINS) are molecules capable of interfering with high-throughput screening results, oftentimes leading to “false hits”. Many of them are

natural polyphenols [1]. Because of their reputation as promiscuous compounds, concerns have been raised when it comes to the pharmaceutical development of natural polyphenols, despite promising bioactivities in cell-free assays against protein targets with therapeutic interest for cancer, diabetes, and Alzheimer's disease, among other pathologies [1,2]. In this work, genistein and resveratrol—two well-known PAINS—were studied in artificial models of the cell membrane, along with phloretin [3], which is a polyphenol that has been extensively used in membrane interaction studies, including dipole potential experiments [4]. By means of di-8-ANEPPS fluorescence ratiometric measurements, we show that these polyphenols act by decreasing the membrane dipole potential, particularly in cholesterol-rich domains such as lipid rafts, which play a key role in important cellular processes [3]. These results suggest a plausible mechanism to which these lipophilic polyphenols owe their 'PAINS label' for their ability to disrupt cell membrane homeostasis [4]. In addition, we present the first synthesis of glucosylresveratrol and demonstrate that polyphenol-promoted membrane dipole potential alterations are fully rescued by C-glucosylation. Hence, our work ultimately sets glucosylpolyphenols as leads for drug development without the risk of false-positive results associated with membrane-disrupting effects that are typical of planar lipophilic polyphenols [3]. This communication also covers our hypothesis for the most likely biophysical mechanisms by which the sugar moiety protects the cell membrane from polyphenol-induced dipole potential alterations.

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References

1. Baell, J.B.; Holloway, G.A. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *57*, 2719–2740, https://doi.org/10.1021/jm901137j.
2. Ingólfsson, H.I.; Thakur, P.; Herold, K.F.; Hobart, E.A.; Ramsey, N.B.; Periole, X.; de Jong, D.H.; Zwama, M.; Yilmaz, D.; Hall, K.; et al. Phytochemicals Perturb Membranes and Promiscuously Alter Protein Function. *ACS Chem. Biol.* **2014**, *9*, 1788–1798, https://doi.org/10.1021/cb500086e.
3. de Matos, A.M.; Blázquez-Sánchez, M.T.; Sousa, C.; Oliveira, M.C.; de Almeida, R.F.M.; Rauter, A.P. C-Glucosylation as a Tool for the Prevention of PAINS-induced Membrane Dipole Potential Alterations. *Sci. Rep.* **2021**, *11*, 4443, https://doi.org/10.1038/s41598-021-83032-3.
4. Filipe, H.A.L.; Sousa, C.; Marquês, J.T.; Vila-Viçosa, D.; Granada-Flor, A.; Viana, A.S.; Santos, M.S.C.S.; Machuqueiro, M.; de Almeida, R.F.M. Differential targeting of membrane lipid domains by caffeic acid and its ester derivatives. *Free Radic. Biol. Med.* **2018**, *115*, 232–245, https://doi.org/10.1016/j.freeradbiomed.2017.12.002.

5.28. Flow Chemistry: A Novel Approach for Becker–Adler Reaction

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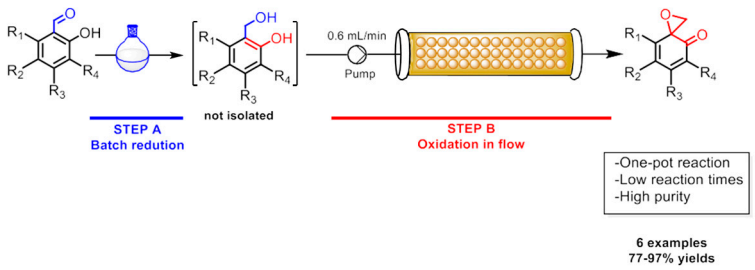
First reported in 1971, the Becker–Adler reaction is the periodate-mediated oxidative dearomatization of salicyl alcohols to spiroepoxydienones [1,2]. Spiroepoxides have a diverse chemical reactivity that allows the synthesis of a diverse array of compounds with different chemical structures. These compounds are useful as intermediates in the synthetic routes of several natural compounds such as tropolones and (−)-4-hydroxyzinowol [3,4].

Nowadays, flow reactions are attracting interest due to their numerous advantages, such as higher safety, better mixing, more efficient heat transfer, and easy scale-up [5]. In a way to improve the Becker–Adler efficiency, we have developed a continuous flow methodology where the reaction promotor is immobilized in the solid phase, and the substrate is passed through in the mobile phase. The continuous flow method allows us to obtain higher yields when compared with batch reactions, and it was possible to recycle the reaction promotor (Scheme 1) [6].



Scheme 1. Becker–Adler reaction in flow conditions.

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References

1. Becker, H.-D.; Bremholt, T.; Adler, E. Oxidative Formation and Photochemical Isomerization of Spiro- Epoxy-2,4-Cyclohexadienones. *Tetrahedron Lett.* **1972**, *13*, 4205–4208.
2. Adler, E.; Holmberg, K.; Ryrfors, L.O. Periodate Oxidation of Phenols. XIV. Oxidation of p-Hydroxybenzyl Alcohol with Periodate and Bismuthate. *Acta Chim. Sinica.* **1974**, *28B*, 883–887, https://doi.org/10.3891/acta.chem.scand.28b-0883.
3. Mitra, P.; Behera, B.; Maiti, T.K.; Mal, D. Angucycline C5 glycosides: regio- and stereocontrolled synthesis and cytotoxicity. *J. Org. Chem.* **2013**, *78*, 9748–9757.
4. Todoroki, H.; Iwatsu, M.; Urabe, D.; Inoue, M. Total Synthesis of (−)-4-Hydroxyzinowol. *J. Org. Chem.* **2014**, *79*, 8835–8849, https://doi.org/10.1021/jo501666x.
5. Plutschack, M.B.; Pieber, B.; Gilmore, K.; Seeberger, P.H. The Hitchhiker's Guide to Flow Chemistry(II). *Chem. Rev.* **2017**, *117*, 11796–11893.
6. Rosatella, A.A.; Afonso, C.A.M. One-Pot Transformation of Salicylaldehydes to Spiroepoxydienones *via* the Adler–Becker Reaction in a Continuous Flow. *ACS Omega* **2022**, *7*, 11570–11577, https://doi.org/10.1021/acsomega.1c05559.

5.29. Development of Porphyrin-Doped Photoactive and Adsorbent Materials

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The particular attention given by the scientific community to porphyrin derivatives is associated with the unique physical, chemical, and biological features of these macrocycles, which are responsible for their success in a wide range of applications, such as solar cells, (chemo)sensors, supramolecular chemistry, and medicine [1]. The increasing resistance of microorganisms to antibiotics and other chemotherapeutics led to the development of new clinical antimicrobial approaches to avoid infections. Antimicrobial photodynamic therapy (aPDT) is pointed out as one of the most promising approaches as an alternative to disable a broad spectrum of pathogenic microorganisms, including those that are highly resistant to conventional antimicrobials [2,3]. In addition, water pollution due to potentially toxic metals is a severe threat to the global ecosystem due to their toxicity and resistance to biodegradation [4]. *Meso*-tetraarylporphyrins display unique photosensitizer and binding properties which can be tailored and potentially improved by incorporation into organic/inorganic materials [5–8].

In this communication, we will discuss the preparation of new materials doped with porphyrin derivatives bearing appropriate functional units to obtain new photoactive materials with bactericidal or chelating properties to be used in photodynamic processes or on the adsorption of several potentially toxic metals from aqueous solution.

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References

1. Kadish, K.M., Smith, K.M., Guilard, R. (Eds.) *Handbook of Porphyrin Science: Volumes 1–12*; World Scientific Publishing Company Co.: Singapore, 2010.
2. Huang, L.; El-Hussein, A.; Xuan, W.; Hamblin, M.R. Potentiation by potassium iodide reveals that the anionic porphyrin TPPS4 is a surprisingly effective photosensitizer for antimicrobial photodynamic inactivation. *J. Photochem. Photobiol. B Biol.* **2018**, *178*, 277–286, https://doi.org/10.1016/j.jphotobiol.2017.10.036.
3. Lan, M.; Zhao, S.; Liu, W., Lee; C.-S.; Zhang, W.; Wang, P. Photosensitizers for photodynamic therapy. *Adv. Healthc. Mater.* **2019**, *8*, 1900132, https://doi.org/10.1002/adhm.201900132.
4. Rathi, B.S.; Kumar, P.S.; Vo, D.-V.N. Critical review on hazardous pollutants in water environment: Occurrence, monitoring, fate, removal technologies and risk assessment. *Sci. Total Environ.* **2021**, *797*, 149134, https://doi.org/10.1016/j.scitotenv.2021.149134.
5. Castro, K.A.; Moura, N.M.; Simões, M.M.; Cavaleiro, J.A.; Faustino, M.D.A.F.; Cunha, Â.; Paz, F.A.A.; Mendes, R.F.; Almeida, A.; Freire, C.S.; et al. Synthesis and characterization of photoactive porphyrin and poly(2-hydroxyethyl methacrylate) based materials with bactericidal properties. *Appl. Mater. Today* **2019**, *16*, 332–341, https://doi.org/10.1016/j.apmt.2019.06.010.
6. Castro, K.A.D.F.; Moura, N.M.M.; Figueira, F.; Ferreira, R.I.; Simões, M.M.Q.; Cavaleiro, J.A.S.; Faustino, M.A.F.; Silvestre, A.J.D.; Freire, C.S.R.; Tomé, J.P.C.; et al. New Materials Based on Cationic Porphyrins Conjugated to Chitosan or Titanium Dioxide: Synthesis, Characterization and Antimicrobial Efficacy. *Int. J. Mol. Sci.* **2019**, *20*, 2522, https://doi.org/10.3390/ijms20102522.
7. Radi, S.; Abiad, C.E.; Moura, N.M.M.; Faustino, M.A.F.; Neves, M.G.P.M.S. New hybrid adsorbent based on porphyrin functionalized silica for heavy metals removal: Synthesis, characterization, isotherms, kinetics and thermodynamics studies. *J. Hazard. Mater.* **2019**, *370*, 80–90, https://doi.org/10.1016/j.jhazmat.2017.10.058.

8. El Abiad, C.; Radi, S.; Faustino, M.A.F.; Neves, M.G.P.M.S.; Moura, N.M.M. Supramolecular Hybrid Material Based on Engineering Porphyrin Hosts for an Efficient Elimination of Lead(II) from Aquatic Medium. *Molecules* **2019**, *24*, 669. https://doi.org/10.3390/molecules24040669.

5.30. Liposomes as a Tool to Potentiate the Antitumor Effect of a New Hybrid Molecule: In Vitro and In Vivo Studies

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Cutaneous melanoma is a very threatening type of cancer, and chemotherapy is the most used strategy to fight it, notwithstanding its lack of selectivity and severe side effects associated. In this sense, the main purpose of the present work was to test new synthesized hybrid molecules, designed as triazene 4-mercaptophenol derivatives (TMD), that possess a dual action conferred by the two pharmacophores: a tyrosine analogue, the substrate for tyrosinase, the enzyme overexpressed in melanoma cells; and a triazene derivative, the cytotoxic agent. Following an in vitro screening in human and murine melanoma cell lines, for the TMD compounds that displayed higher antiproliferative properties, cell cycle distribution and hemolytic activity were also evaluated [1]. For the most promising compound, aiming to improve its in vivo fate, reduce systemic toxicities, and promote an accumulation at melanoma tumor sites, its incorporation in long blood-circulating liposomes, using a dehydration–rehydration method followed by an extrusion step to reduce and homogenize their mean size, was performed [2]. Liposomal formulations were extensively characterized, presenting a mean size of around 150 nm, a neutral surface charge, an incorporation efficiency superior to 90%, and a stability in suspension higher than 65% one week after preparation. The so optimized TMD liposomal formulation was tested in vivo in terms of safety and antitumor effect [3]. The latter was evaluated in a syngeneic C57BL/6 melanoma murine model, after a subcutaneous injection of B16F10 cells. The progression of the tumor volume was assessed for animals treated with TMD in free and liposomal forms and compared with induced and non-treated mice (negative control) or mice treated with dacarbazine (positive control), which is a drug in clinical use for the treatment of metastatic melanoma. TMD in the liposomal form displayed the highest antitumor effect as revealed by the relative tumor volume (RTV) and the lowest average mass tumor at the end of the experimental protocol. No toxic side effects were observed for mice treated with TMD formulations based on tissue index in vital organs or hepatic enzyme levels. Overall, TMD liposomes demonstrated their therapeutic potential against melanoma. The rational of the present study is depicted in Figure 1.

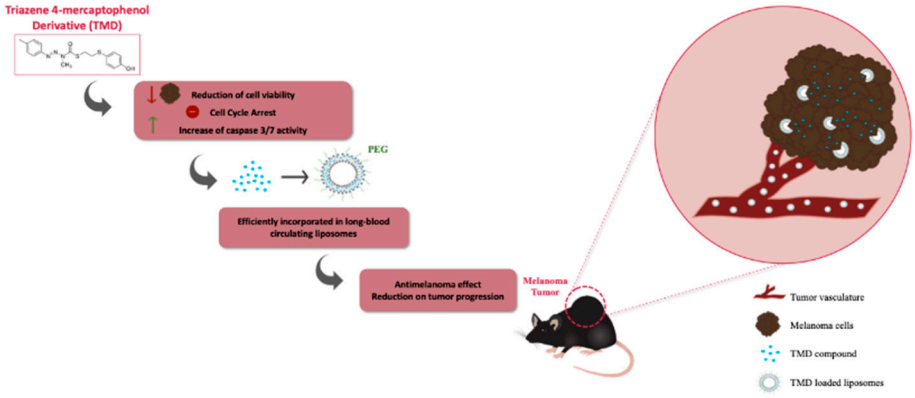

Figure 1. Schematic representation of the present study.

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References

1. Cruz, N.; Pinho, J.O.; Soveral, G.; Ascensão, L.; Matela, N.; Reis, C.; Gaspar, M.M. A Novel Hybrid Nanosystem Integrating Cytotoxic and Magnetic Properties as a Tool to Potentiate Melanoma Therapy. *Nanomaterials* **2020**, *10*, 693. https://doi.org/10.3390/nano10040693.
2. Calado S.; Eleutério, C.; Mendes, E.; Rocha, M.J.; Francisco, A.P.; Gaspar M.M.J. Nanoformulations of a Triazene Analogue with Specific Affinity to Human Melanoma. *J. Nanosci. Adv. Tech.* **2016**, *1*, 1–9, https://doi.org/10.24218/jnat.2016.16.
3. O Pinho, J.; Amaral, J.D.; E Castro, R.; Rodrigues, C.M.; Casini, A.; Soveral, G.; Gaspar, M.M. Copper complex nanoformulations featuring highly promising therapeutic potential in murine melanoma models. *Nanomedicine* **2019**, *14*, 835–850, https://doi.org/10.2217/nnm-2018-0388.

5.31. Synthesis of Chromeno-Fused Systems via Lewis Base-Dependent [3 + 2] or [3 + 3] Annulation Reactions of Allenic Esters and 3-Nitro-2H-Chromenes

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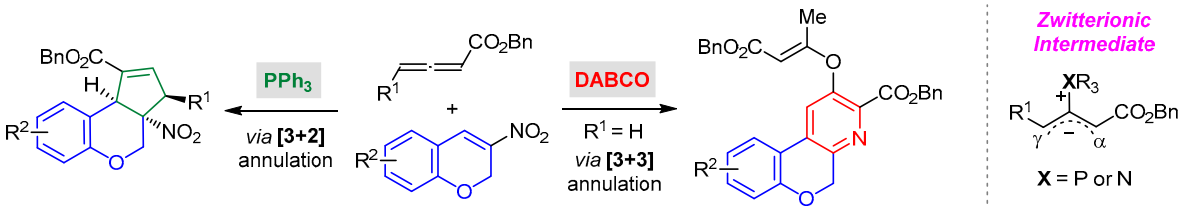
The demand for efficient and selective methods for the synthesis of polycyclic systems remains a topic of great interest in organic synthesis and Medicinal Chemistry, since these structures are found in the core of several natural and synthetic compounds with biological activity. Among them, molecules incorporating the chromene scaffold are well-known for their pharmacological activities (e.g., antimicrobial, anti-inflammatory, anttumoral) [1–3].

Allenic esters (allenoates) are attractive building blocks, as their chemical behavior can be modulated by selection of the appropriate Lewis base (LB) catalyst. The zwitterionic intermediate, generated by the addition of a LB to the allenoate's β-carbon, can react differently with electrophiles depending on the nature of the LB. Under phosphine catalysis, [3 + 2] annulation products are obtained, whereas in the presence of tertiary amines, conjugate addition may be observed for activated alkenes. We envisaged that these allenoates' reactivity features could be explored to carry out reactions with 3-nitro-2*H*-chromenes as an approach to new and diverse chromene-fused derivatives. Herein, we described studies on the Lewis base-catalyzed [3 + 2] and [3 + 3] annulation reactions of allenoates and 3-

nitro-2*H*-chromenes. Under PPh_3-catalyzed conditions, tetrahydrocyclopenta[*c*]chromenes were obtained via formal [3 + 2] cycloaddition [4], while the DABCO-catalyzed reaction furnished 5*H*-chromeno[3,4-*b*]pyridines incorporating two allenoate units via a [3 + 3] annulation reaction [5] (Scheme 1).



Scheme 1. Phosphine- and DABCO-catalyzed reactions of 3-nitro-2*H*-chromenes and allenoates.

Funding: Thanks are due to the Coimbra Chemistry Centre (CQC), which is supported by the Portuguese Agency for Scientific Research "Fundação para a Ciência e a Tecnologia" (FCT), through project UIDB/00313/2020 and UIDP/00313/2020, co-funded by COMPETE2020-UE. The authors also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Patil, S.A.; Patil, R; Pfeffer, L.M.; Miller, D.D. Chromenes: Potential new chemotherapeutic agents for cancer. *Fut. Med. Chem.* **2013**, *5*, 1647–1660, https://doi.org/10.4155/fmc.13.126.
2. Pratap, R.; Ram, V.J. ChemInform Abstract: Natural and Synthetic Chromenes, Fused Chromenes, and Versatility of Dihydrobenzo[h]chromenes in Organic Synthesis. *ChemInform* **2014**, *46*, https://doi.org/10.1002/chin.201503281.
3. Costa, M.; Dias, T.A.; Brito, A.; Proença, F. Biological importance of structurally diversified chromenes. *Eur. J. Med. Chem.* **2016**, *123*, 487–507, https://doi.org/10.1016/j.ejmech.2016.07.057.
4. Soares, M.I.L.; Gomes, C.S.B.; Nunes, S.C.C.; Pais, A.A.C.C.; Pinho e Melo, T.M.V.D. Phosphane-catalyzed [3+2] annulation of allenoates with 3-nitro-2H-chromenes: Synthesis of tetrahydrocyclopenta[c]chromenes. *Eur. J. Org. Chem.* **2019**, *2019*, 5441–5451.
5. Soares, M.I.L.; Gomes, C.S.B.; Oliveira, M.C.; Marçalo, J.; Pinho e Melo, T.M.V.D. Synthesis of 5H-chromeno[3,4-b]pyridines via DABCO-catalyzed [3+3] annulation of 3-nitro-2H-chromenes and allenoates. *Org. Biom. Chem.* **2021**, *19*, 9711–9722.

5.32. Multivalent NHS-Activated Acrylates for Orthogonal Site-Selective Functionalization of Peptides at Cysteine Residues

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Traditional chemotherapeutic drugs with strong cytotoxicity face long-standing problems regarding non-specific biodistribution and targeting in the body, poor water solubility and low therapeutic indices [1].

Due to their biological activity, many peptides and proteins are known to be potent anticancer agents. The development of antibody–drug conjugate (ADC) therapy has emerged with worldwide marketing approvals for oncotherapy due to the advances in solid-phase peptide synthesis, recombinant DNA and hybridoma technology that allowed the production of unlimited quantities of clinical grade peptides and proteins [2].

The chemoselectivity and mildness of the processes attained with protein bioconjugation should successfully install modifications at pre-determined sites without disturbing the structure, function and activity of proteins. However, the need for fast kinetics sometimes compromises the selectivity of the process, leading to heterogeneous conjugates. A way to surpass this is to apply site-selective methods that result in homogeneous products by promoting such reactions under natural biological conditions targeting low relative abundant cysteine residues [2].

Our research group started to study NHS-activated acrylic ester as suitable reagents for the selective stapling of amino-sulfhydryl groups [3]. After achieving wonderful results, we decided to broaden the research by designing novel NHS-activated acrylates that hold various payloads in a single bioconjugation handle and can site-selectively and orthogonally target the N terminal cysteine of peptides. The bioconjugation generates a stable 1,4 thiazepan 5-one core, and the attained bioconjugates were designed to be further used for theranostics studies (Figure 1).

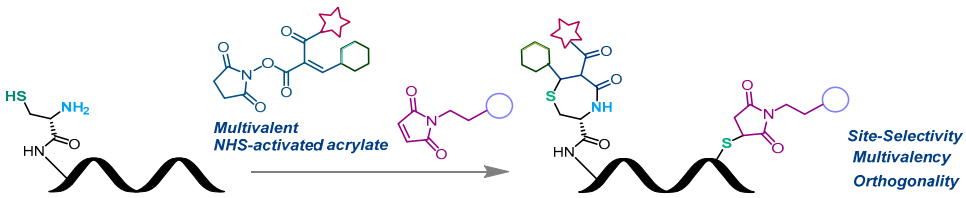

Figure 1. Multivalent NHS-activated acrylate for the site-selective and orthogonal multifunctionalization of peptides.

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References

1. Lu, J.; Jiang, F.; Lu, A.; Zhang, G. Linkers Having a Crucial Role in Antibody–Drug Conjugates. *Int. J. Mol. Sci.* **2016**, *17*, 561, https://doi.org/10.3390/ijms17040561.
2. Hoyt, E.A.; Cal, P.M.S.D.; Oliveira, B.L.; Bernardes, G.J.L. Contemporary approaches to site-selective protein modification. *Nat. Rev. Chem.* **2019**, *3*, 147–171.
3. Silva, M.J.S.A.; Faustino, H.; Coelho, J.A.S.; Pinto, M.V.; Fernandes, A.; Compañón, I.; Corzana, F., Gasser, G.; Góis, P.M.P. Efficient Amino-Sulfhydryl Stapling on Peptides and Proteins Using Bifunctional NHS-Activated Acrylamides. *Angew. Chem. Int. Ed.* **2021**, *60*, 10850–10857.

5.33. Liposomes Incorporating a Novel Metal-Complex: An Alternative and Effective Treatment against Colorectal Cancer

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Colorectal cancer (CRC) is the third most incident malignancy and second cause of cancer-related mortality. Current therapies rely on chemotherapeutic agents with inefficient biodistribution profiles and poor specificity. Cisplatin brought attention to the potential of metal-based complexes (1). However, these compounds present low solubility and are associated to toxic side effects due to low specificity. Liposomes can overcome these drawbacks, improving its solubility, therapeutic efficacy and reducing toxicity. In fact, liposomes can remain in circulation for long periods of time, allowing extravasation and accumulation at tumor sites. Additionally, the microenvironment of solid tumors is slightly acidic, contrasting with healthy tissues. Liposomes can include lipids capable of inducing the release of loaded compounds when in acidic sites (2,3). The objective of this project was to develop a lipid-based system with long blood circulating times and pH-sensitive properties that can incorporate a novel metal-based complex to passively target tumor sites.

Various metal-based complexes were screened in vitro using murine and human colon cancer cell lines. Liposomal formulations of the most promising compound, a zinc-based complex, were developed and characterized in terms of incorporation efficiency (I.E.), size and surface charge. The antiproliferative effect of the selected zinc complex toward colon cancer cell lines cultured in 2D or 3D settings was assessed both in free and liposomal forms. Additionally, internalization studies of rhodamine-labeled liposomes into 3D spheroids of colon cancer cell lines were performed by confocal microscopy. Lastly, the efficacy of the zinc-based complex was assessed in vivo in a syngeneic murine colon cancer model (3).

The zinc-based complex was efficiently incorporated in liposomes, with an I.E. of 76% and a mean size under 120 nm. In a 2D setting, the complex displayed an IC50 below 15 μM in its free and liposomal forms. In a 3D setting, higher concentrations were needed to achieve antiproliferative properties, and liposome internalization was time and concentration dependent. In vivo studies showed that liposomal formulations of the zinc-based complex displayed a similar antitumor effect than 5-FU (positive control) using a therapeutic dose three-fold lower.

This project is an important step toward the development of more effective therapeutic strategies for CRC.

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References

1. Matos, C.P.; Addis, Y.; Nunes, P.; Barroso, S.; Alho, I.; Martins, M.; Matos, A.P.; Marques, F.; Cavaco, I.; Pessoa, J.C.; et al. Exploring the cytotoxic activity of new phenanthroline salicylaldimine Zn(II) complexes. *J. Inorg. Biochem.* **2019**, *198*, 110727, https://doi.org/10.1016/j.jinorgbio.2019.110727.
2. Farinha, P.; Pinho, J.O.; Matias, M.; Gaspar, M.M. Nanomedicines in the treatment of colon cancer: a focus on metallodrugs. *Drug Deliv. Transl. Res.* **2021**, *12*, 49–66, https://doi.org/10.1007/s13346-021-00916-7.
3. Pinho, J.O.; da Silva, I.V.; Amaral, J.D.; Rodrigues, C.M.P.; Casini, A.; Soveral, G.; Gaspar, M.M. Therapeutic potential of a copper complex loaded in pH-sensitive long circulating liposomes for colon cancer management. *Int J. Pharm.* **2021**, *599*, 120463, https://doi.org/10.1016/j.ijpharm.2021.120463.

5.34. Manipulation of Organic Molecules by Vibrational Excitation with Near-Infrared Light

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Infrared vibrational excitation is a promising approach to achieve the controlled manipulation of organic molecules in ways that cannot be attained via thermal or electronic excitation. The possibility of selectively manipulating a chosen molecule in a complex mixture, including the manipulation of a specific conformation existing in a particular environment, has been demonstrated in conjugation with the matrix isolation technique (i.e., with a sample trapped in a solidified noble-gas at ≈ 10 K). In this context, narrowband near-IR light is applied to selectively deposit energy in a vibrational state (typically a stretching overtone mode) of a molecular target [1]. So far, such an approach has been applied to induce conformational isomerizations of different molecular fragments, such as −OH, −SH, −OMe, −CHO and $-CH_2OH$ [2].

In recent breakthrough investigations, we have demonstrated that besides conformation isomerizations, molecular reactions involving bond breaking and bond forming can also be activated by infrared vibrational excitation under matrix isolation conditions [3–5]. Herein, we will present two pioneer examples: (i) the infrared-induced bidirectional tautomerization of thiotropolone; (ii) the infrared-induced electrocyclic ring expansion of a benzazirine to a cyclic ketenimine (Figure 1). This accomplishment opens the door for harnessing IR vibrational excitation as a tool to guide a variety of molecular structure manipulations in an unprecedented highly selective manner and for developing new cutting-edge strategies to quest fundamental scientific questions and practical applications.

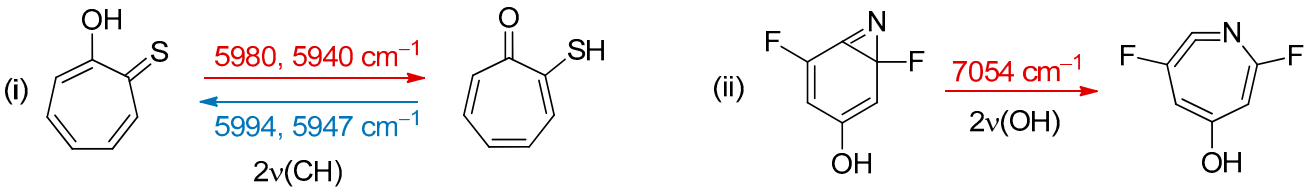

Figure 1. Proof-of-principle examples of molecular reactions induced under matrix isolation conditions by selective vibrational excitation with near-IR light at the frequency of stretching overtones (2ν(CH) and 2ν(OH)).

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References

1. Nunes, C.M.; Reva, I.; Fausto, R. Conformational isomerizations triggered by vibrational excitation of second stretching overtones. *Phys. Chem. Chem. Phys.* **2019**, *21*, 24993–25001, https://doi.org/10.1039/c9cp05070a.
2. Jesus, A.J.L.; Nunes, C.M.; Fausto, R.; Reva, I. Conformational control over an aldehyde fragment by selective vibrational excitation of interchangeable remote antennas. *Chem. Commun.* **2018**, *54*, 4778–4781, https://doi.org/10.1039/c8cc01052h.
3. Nunes, C.M.; Pereira, N.A.M.; Reva, I.; Amado, P.; Cristiano, M.L.S.; Fausto, R. Bond-Breaking/Bond-Forming Reactions by Vibrational Excitation: Infrared-Induced Bidirectional Tautomerization of Matrix-Isolated Thiotropolone. *J. Phys. Chem. Lett.* **2020**, *11*, https://doi.org/10.1021/acs.jpclett.0c02272.

4. Pereira, N.A.M.; Nunes, C.M.; Reva, I.; Fausto, R. Evidence of IR-Induced Chemistry in a Neat Solid: Tautomerization of Thiotropolone by Thermal, Electronic, and Vibrational Excitations. *J. Phys. Chem. A* **2021**, *125*, 6394–6403, https://doi.org/10.1021/acs.jpca.1c04081.
5. Nunes, C.M.; Pereira, N.A.M.; Viegas, L.P.; e Melo, T.M.V.D.P.; Fausto, R. Inducing molecular reactions by selective vibrational excitation of a remote antenna with near-infrared light. *Chem. Commun.* **2021**, *57*, 9570–9573, https://doi.org/10.1039/d1cc03574f.

5.34.1. Targeting Breast Cancer through PKC Modulation with Abietane Royleanone Derivatives

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Cancer continues to be one of the leading causes of death worldwide. Protein kinase C family is an attractive target for cancer therapy and PKC α, δ, ε, and ζ deserve special attention in breast cancer research [1]. *Plectranthus* spp. (Lamiaceae) are a well-known source of interesting abietanes, such as the cytotoxicity abietane diterpenoid 7α-acetoxy-6β-hydroxyroyleanone (Roy, Figure 1). Research suggest that Roy can be an interesting lead molecule for future drug development. Furthermore, Roy can be obtained in high amounts from *P. grandidentatus* [2].

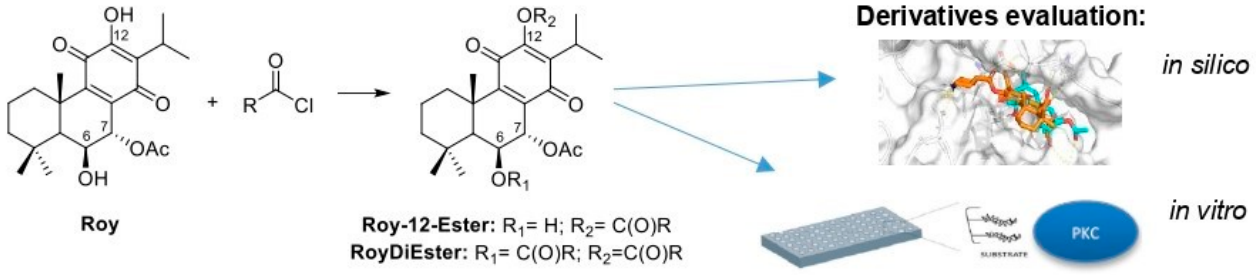

Figure 1. Preparation and evaluation of Roy derivatives.

The aim of this study was to obtain Roy and derivatize it to improve its cytotoxic properties, focusing on PKC modulation for breast cancer (Figure 1). Therefore, the reactional conditions to prepare ester derivatives were studied. In addition, a library of new theoretical 12-OH Roy ester derivatives was studied via molecular docking in PKC isoforms (α, δ, ε, and ζ).

The acetonic ultrasonic-assisted extraction of P. grandidentatus (yield of 2.3%, *w*/*w*) afforded 1 g (≈ 0.04%, *w*/*w*) of pure Roy. Reactivity study pointed to the 12-OH position as the most reactive for esterification (Figure 1). Roy-12-ester derivatives were obtained using mild conditions, with overall good yields (33–86%). For both positions' derivatization, results suggested an excess of reagents, high temperature (50 °C), and higher reaction time. Molecular docking screening presented some promising derivatives, which were

predicted to bind in a similar location and strength as known active compounds. New ester hit derivatives are currently in preparation to be further evaluated as PKC modulators.

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References

1. Isca, V.M.S.; Sencanski, M.; Filipovic, N.; Dos Santos, D.J.V.A.; Gašparović, A..; Saraíva, L.; Afonso, C.A.M.; Rijo, P.; García-Sosa, A.T. Activity to Breast Cancer Cell Lines of Different Malignancy and Predicted Interaction with Protein Kinase C Isoforms of Royleanones. *Int. J. Mol. Sci.* **2020**, *21*, 3671, https://doi.org/10.3390/ijms21103671.
2. Bernardes, C.E.S.; Garcia, C.; Pereira, F.; Mota, J.; Pereira, P.; Cebola, M.J.; Reis, C.P.; Correia, I.; Piedade, M.F.M.; da Piedade, M.E.M.; et al. Extraction Optimization and Structural and Thermal Characterization of the Antimicrobial Abietane 7α-Acetoxy-6β-hydroxyroyleanone. *Mol. Pharm.* **2018**, *15*, 1412–1419, https://doi.org/10.1021/acs.molpharmaceut.7b00892.

5.35. Understanding the Molecular Interactions between Protein Models and Phenolic Compounds

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To respond to the sharp increase in the world population, the agri-food sector is now starting to implement cleaner, and sustainable approaches. One of the strategies adopted was the use of industrial agri-residues such as stalks, leaves, bark, roots, bagasse, and seeds, revealing themselves as a source of bioactive compounds. Phenolic compounds (PCs) are known for their antioxidant, anti-inflammatory, anticancer and antiaging properties [1]. Alongside the health-promoting effects, PCs have been described as able to modulate the main organoleptic characteristics of plant-derived foods and beverages [2]. Moreover, their natural ability to bind to proteins can bring new insights in the use of PC as emulsifier agents [3]. One of the current trends in the sector is the replacement of animal-derived proteins by vegetable alternatives. In this study, the molecular perspective of the use of PCs as emulsifiers has been studied in a yeast protein extract (YPE)-based mayonnaise in comparison with the traditional egg-derived mayonnaise Thus, the molecular mechanisms of the interaction between egg or YPE protein models and PCs (gallic acid—GA, tannic acid—TA, onion extract—OE, blueberry extract—BE and grape seed extract—GSE) were unraveled by fluorescence quenching. The molecular binding models were studied at pH 7.4 (biological conditions) and at pH 3.5 (mayonnaise conditions) and at different temperatures (4 °C and RT), simulating the storage conditions.

Overall, different mechanisms of molecular interaction were found for the different PCs. Molecular affinity constants were calculated by using the Stern–Volmer equation. A general trend to higher constant affinity was observed in the YPE model when compared to egg proteins. The PCs were found to be the main factor affecting the affinities; these also depended on the temperature and the pH. The results obtained within this study clearly showed the potential of PC to be used as natural emulsifiers, which can conquer the food industry in response to the consumer demand for clean labeling and potentially health-beneficial foods. However, future studies are required to understand the structure/activity relationships and main dose/response behaviors.

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References

1. Perez-Gregorio, R.; Simal-Gandara, J. A Critical Review of Bioactive Food Components, and of their Functional Mechanisms, Biological Effects and Health Outcomes. *Curr. Pharm. Des.* **2017**, *23*, 2731–2741, https://doi.org/10.2174/1381612823666170317122913.
2. Soares, S.; Kohl, S.; Thalmann, S.; Mateus, N.; Meyerhof, W.; De Freitas, V. Different Phenolic Compounds Activate Distinct Human Bitter Taste Receptors. *J. Agric. Food Chem.* **2013**, *61*, 1525–1533, https://doi.org/10.1021/jf304198k.
3. Perez-Gregorio, M.R.; Simal-Gandara, J. A Critical Review of the Characterization of Polyphenol–Protein Interactions and of Their Potential Use for Improving Food Quality. *Curr. Pharm. Des.* **2017,** *23*, 2742–2753, https://doi.org/10.2174/1381612823666170202112530.

6. Flash Presentations

6.1. Dual Inhibitors for Multiple Myeloma—A Computational Campaign

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Multiple myeloma in the United States (US) alone accounts 1.8% of all new cancer cases in 2020, with an estimated 32,270 new cases. In mid-2020, the number of deaths as a result of multiple myeloma (MM) was already 12,830, representing 2.1% of all cancer deaths. In the US, there were an estimated 140,779 people living with multiple myeloma, in 2017 [1]. Recent studies revealed that relapse of myeloma developed due to the acquisition of resistance to proteasome inhibitors, owing to mutations of proteasome complex, upregulation of transporter channels, or cytochrome components, and induction of alternative compensatory pathways [2]. Proteasomes are large, multicatalytic protein complexes that cleave cellular proteins into peptides. Proteasome inhibitors are an important class of drugs for the treatment of multiple myeloma and mantle cell lymphoma, and they are being investigated for other diseases [3]. The key nuclear export protein CRM1/XPO1 may represent a promising novel therapeutic target in human MM. Here, we showed that chromosome region maintenance 1 (CRM1) is highly expressed in patients with MM, plasma cell leukemia cells and increased in patient cells resistant to bortezomib treatment [4].

In this work, we propose a multitarget approach in which we employ computational strategies to identify dual proteasome and CRM1 inhibitors that could overcome resistance in MM and other cancers. We created 3D-pharmacophore models, using MOE2020 software to support hit finding. Pharmacophore models were made for both proteasome and CRM1 targets. Molecular docking was performed in both models to predict possible dual inhibitors. The performance of all models was validated against robust databases, and the

most predictive models were optimized further by systematic modification of the chemical features.

The results revealed valuable information about the key interactions and the 3D geometries associated with the proteasome and CRM1 dual inhibition activity.

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References

1. National Cancer Institute. SEER Cancer Stat Facts. Available online: https://seer.cancer.gov/statfacts/html/mulmy.html (Accessed on 14 June 2022).
2. Soriano, G.P.; Besse, L.; Li, N.; Kraus, M.; Besse, A.; Meeuwenoord, N.; Bader, J.; Everts, B.; den Dulk, H.; Overkleeft, H.S.; et al. Proteasome Inhibitor-Adapted Myeloma Cells Are Largely Independent from Proteasome Activity and Show Complex Proteomic Changes, in Particular in Redox and Energy Metabolism. *Leukemia* **2016**, *30*, 2198–2207, https://doi.org/10.1038/leu.2016.102.
3. Fricker, L.D. Proteasome Inhibitor Drugs. *Annu. Rev. Pharmacol. Toxicol.* **2020**, *60*, 457–476, https://doi.org/10.1146/annurev-pharmtox-010919-023603.
4. Tai, Y.-T.; Landesman, Y.; Acharya, C.; Calle, Y.; Zhong, M.Y.; Cea, M.; Tannenbaum, D.; Cagnetta, A.; Reagan, M.; Munshi, A.A.; et al. CRM1 inhibition induces tumor cell cytotoxicity and impairs osteoclastogenesis in multiple myeloma: molecular mechanisms and therapeutic implications. *Leukemia*. **2014**, *28*, 155–165, https://doi.org/10.1038/leu.2013.115.

6.2. Synthesis and Neuroprotection Studies of New Tricyclic Compounds

Márcia S. Martins [1,2], Miguel Maia [1,2], Eva Gil-Martins [3], Luís Gales [4,5], Fernando Remião [3], Madalena M. M. Pinto [1,2], Renata Silva [3,*] and Emília Sousa [1,2,*]

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The enzymes β-secretase 1 (BACE-1) and glycogen synthase kinase 3β (GSK-3β) are involved in the considered major pathological pathways of Alzheimer's disease (AD). To stop the progression of this disease, the creation of a multitarget-direct ligand (MTDL) is an upcoming strategy [1,2]. The main goal of this work was the synthesis of potential dual BACE-1/GSK-3β inhibitors and the evaluation of their neuroprotective properties in vitro.

To reach the synthesis of MTDLs, presented in Figure 1, the conjugation of a tricyclic derivative, with aliphatic and aromatic amines was performed, resulting in the synthesis of eight amides. Two amines were successfully synthetized and purified through the reduction in the corresponding amides. In total, ten compounds were obtained with yields between 18% and 95%.

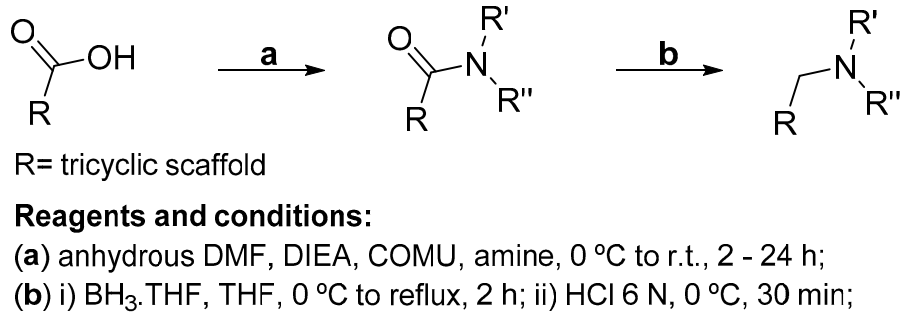

Figure 1. General synthesis of the tricyclic derivatives.

Their neuroprotective properties were evaluated using differentiated SH-SY5Y cells, and several compounds demonstrated potential to protect the cells against the cytotoxicity induced by three chemical aggressors, MPP^+, iron (III), and β-amyloid (Aβ) peptide, as well as potential to activate P-glycoprotein (P-gp). The compounds with halogen atoms in their structure demonstrated a higher protection against iron (III)-induced cytotoxicity, which was not dependent on iron chelation. Neuroprotective effects against $A\beta_{1-42}$-induced cytotoxicity were shown to be dependent on P-gp activation.

In conclusion, a small library of tricyclic derivatives was successfully obtained and characterized by the neuroprotective properties of several compounds with potential for the treatment of neurodegenerative diseases, namely AD and Parkinson's disease.

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References

1. Maia, M.A.; Sousa, E. BACE-1 and γ-Secretase as Therapeutic Targets for Alzheimer's Disease. *Pharmaceuticals* **2019**, *12*, 41, https://doi.org/10.3390/ph12010041.
2. Martins, M.; Silva, R.; M, M.M.P.; Sousa, E. Marine Natural Products, Multitarget Therapy and Repurposed Agents in Alzheimer's Disease. *Pharmaceuticals* **2020**, *13*, 242, https://doi.org/10.3390/ph13090242.

6.3. Phenanthroline-Based Derivatives as G-Quadruplex pre-MIR150 Binders: Synthesis and Evaluation

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The human MIR150 cells are significantly upregulated in Non-Small Cell Lung Cancer (NSCLC) and have been reported to have an important role in NSCLC development [1,2]. Thus, the control of mature MIR150 production can provide a strategy to fight NSCLC development. The presence of G-quadruplex (G4) can affect their recognition and consequent processing by Dicer [3]. Recently, it has been reported that pre-MIR150 folds

into a G4) structure [4], which could regulate their levels, thus unveiling a new potential therapeutic strategy. The formation of G4 structures in the stem-loop region of pre-miRNAs can interfere with Dicer activity and decrease mature miRNA production inside the cell [5].

In this context, we have synthetized different imines and amides, derivatives of phenanthroline with the aim of binding and stabilizing the G4 motif found in the region of pre-MIR150. The interaction of these ligands with the G4 motif has been evaluated using a combination of biophysical methods and showed moderate activity in terms of thermal stabilization.

This study has explored the suitability of the synthesized molecules to interact with the G4 motif and provides invaluable information about the structural modifications that should be carried out in order to maximize their activity.

Funding: Joana Figueiredo acknowledges a doctoral fellowship grant from the FCT ref. SFRH/BD/145106/2019 and COST Action CA17140 Nanomedicine—from the bench to the bedside (NANO2CLINIC) for the Short-Term Scientific Mission (STSM) grant (ECOST-STSM-Request-CA17140-47763).

References

1. Jiang, K.; Shen, M.; Chen, Y.; Xu, W. miR-150 promotes the proliferation and migration of non-small cell lung cancer cells by regulating the SIRT2/JMJD2A signaling pathway. *Oncol. Rep.* **2018**, *40*, 943–951, https://doi.org/10.3892/or.2018.6487.
2. Li, H.; Ouyang, R.; Wang, Z.; Zhou, W.; Chen, H.; Jiang, Y.; Zhang, Y.; Li, H.; Liao, M.; Wang, W.; Ye, M.; et al. MiR-150 promotes cellular metastasis in non-small cell lung cancer by targeting FOXO4. *Sci. Rep.* **2016**, *6*, 39001, https://doi.org/10.1038/srep39001.
3. Mirihana Arachchilage, G.; Dassanayake, A.C.; Basu, S. A Potassium Ion-Dependent RNA Structural Switch Regulates Human Pre-miRNA 92b Maturation. *Chem. Biol.* **2015**, *22*, 262–272, https://doi.org/10.1016/j.chembiol.2014.12.013.
4. Figueiredo, J.; Miranda, A.; Lopes-Nunes, J.; Carvalho, J.; Alexandre, D.; Valente, S.; Mergny, J.-L.; Cruz, C. Targeting nucleolin by RNA G-quadruplex-forming motif. *Biochem. Pharmacol.* **2021**, *189*, 114418, https://doi.org/10.1016/j.bcp.2021.114418.
5. Pandey, S.; Agarwala, P.; Jayaraj, G.G.; Gargallo, R.; Maiti, S. The RNA Stem–Loop to G-Quadruplex Equilibrium Controls Mature MicroRNA Production inside the Cell. *Biochemistry* **2015**, *54*, 7067–7078, https://doi.org/10.1021/acs.biochem.5b00574.

6.4. New Red-Shifted 4-Styryl Coumarins as Potential Fluorescent Labels for Biomolecules

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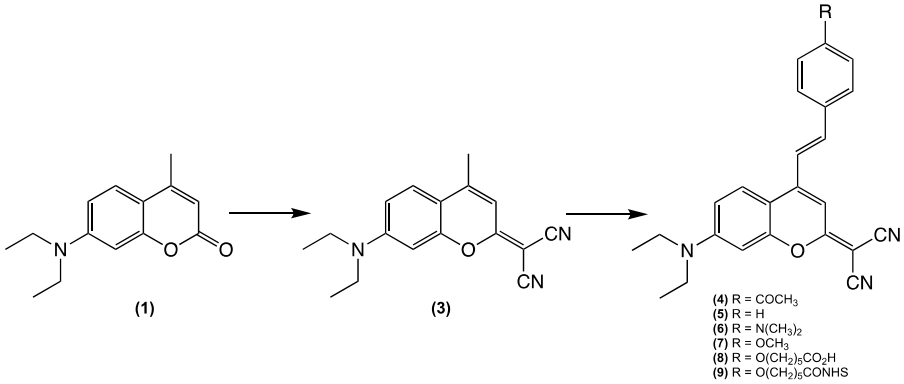
Cellular biology, medicine, pharmacy, environmental sciences and other important scientific areas require highly sensitive analytical techniques to track and detect nucleic acids, oligonucleotides, antibodies, amino acids, proteins, lipids, carbohydrates, and other biomolecules. Of all sensitive analytical techniques, fluorescent labeling presents numerous advantages as it allows the use of small sample quantities as well as the respective fluorescent labels [1,2]. The availability and the development of new fluorophores are now enabling previously impossible studies of cellular processes and the detection of specific components of complex biomolecular assemblies with selectivity and exquisite sensitivity, in vitro and in vivo, as well the analysis of their interactions [3]. In this context, due to

the high cost of the available commercial fluorescent labels, coumarin derivatives can be a solution to develop low-cost new fluorophores with absorption and emission at long wavelengths, combined with large Stokes shifts. In this work, we developed an effective synthetic strategy to produce new red-shifted 4-styryl coumarins using 7-diethylamino-4-methylcoumarin (1) as a starting material. These could be used to produce fluorescent labels for biomolecules. The main synthetic strategy to obtain 4-styryl coumarins was based on the high acidity of the methyl protons present at position 4 in 2-(7-(diethylamino)-4-methyl-2H-chromen-2-ylidene)malononitrile (3), that enable aldol condensation reactions. The mentioned dicyanomethylene-coumarinylmethyl derivative, with a higher bathochromic shift than 100 nm when compared with its precursor, was obtained by the incorporation of two cyano groups in position 2 after the thionation of the carbonyl group of the lactone. With the objective to extend the delocalization of the π-electron system, we have designed and synthesized new 4-styryl coumarin derivatives, with absorption and emission at long wavelengths, combined with large Stokes shifts, using a simple, low-cost and efficient synthetic strategy (Scheme 1).



Scheme 1. Synthesis of the 4-styryl coumarin derivatives.

Funding: The study was performed under the framework of THE SCREAM Project—"Touchstone for Heritage Endangered by Salt Crystallization, a Research Enterprise on the Art of Munch" (ref. FCT-ALT20-03-0145-FEDER-031577) financed by the Portuguese Foundation for Science and Technology (FCT) through National and European Funds.

References

1. Pereira, A.; Martins, S.; Caldeira, A.T. Coumarins as Fluorescent Labels of Biomolecules. In Phytochemicals in Human Health; Rao, V., Mans, D.R.A., Rao, L., Eds.; IntechOpen Limited: London, UK, 2020; Chapters 8, https://doi.org/10.5772/intechopen.85973.
2. Saboo, H. Fluorescent labeling techniques in biomolecules: a flashback. *RSC Adv.* **2012**, *2*, 7017–7029.
3. Fang, X.; Zheng, Y.; Duan, Y.; Liu, Y.; Zhong, W. Recent Advances in Design of Fluorescence-Based Assays for High-Throughput Screening. *Anal. Chem.* **2019**, *91*, 482–504, https://doi.org/10.1021/acs.analchem.8b05303.

6.5. Exploiting the Electron Transport Chain of Mycobacterium Tuberculosis as a Target in the Development of New Anti-Tuberculosis Drugs

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Tuberculosis (TB) is a contagious infection caused by Mycobacterium tuberculosis (Mtb). Prior to the SARS-CoV-2 pandemic, more deaths occurred due to Mtb than due

to any other infectious agent [1]. TB constitutes a significant public health concern, since the global control of this disease is highly challenged due to the extended duration of therapy, patient compliance and the development and spread of multidrug-resistant (MDR) and extensively drug-resistant TB (XDR-TB) [2]. Another challenge is that the available anti-TB drugs fail to address the latent infections. These latent infections are prevalent in 90% of infected people, and when the immune system is compromised, these latent forms can become active and contagious. The discovery of novel molecular structures and the development of new drugs with potent activity against drug-resistant replicant and non-replicant Mtb are, therefore, urgently needed [2].

Mtb's viability depends on the energy produced by its respiratory chain. A combination of compounds targeting different components of the electron transport chain (ETC) has been considered as an innovative and potentially successful approach to avoid the emergence of resistance [3,4].

The aim of this project is to progress a set of pyrroloquinolones (PYQ), which arose from a screening against Mtb H37Rv strain, into viable lead candidates. These compounds are developed to multitarget the ETC of Mtb through the inhibition of cytochrome *bcc* and a simultaneous release of nitric oxide. Here, we present the synthesis of a small library of cytochrome *bcc* inhibitors and hybrids. To expand the library of PYQ, we diversify the linker between the PYQ core and the substituents at the R position (Figure 1). Compounds' biological evaluation against Mtb H37Rv strain as well as solubility determination were performed.

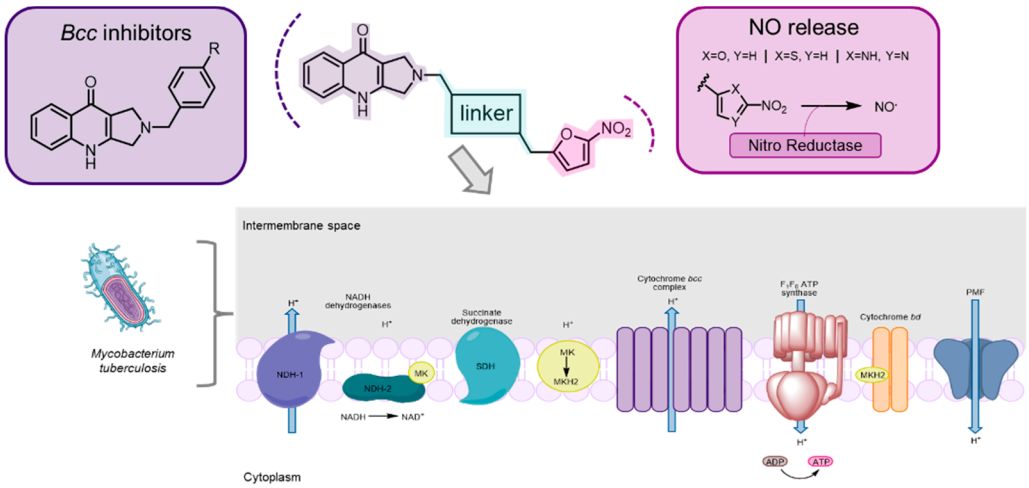

Figure 1. Structure of the anti-TB multitargeting compounds.

Funding: We thank the Fundação para a Ciência e Tecnologia (FCT); this research was funded by projects UIDB/04138/2020 and UIDP/04138/2020 and PTDC/MED-FAR/30266/2017 and LISBOA-01-014-FEDER-030266 (FCT and FEDER). We also acknowledge the FCT for fellowship 2020.05735.BD (M.C.).

References

1. World Health Organization. *Global Tuberculosis Report 2021*; World Health Organization: Geneva, Switzerland, 2021; ISBN: 978-92-4-003702-1.
2. Campaniço, A.; Harjivan, S.G.; Warner, D.F.; Moreira, R.; Lopes, F. Addressing Latent Tuberculosis: New Advances in Mimicking the Disease, Discovering Key Targets, and Designing Hit Compounds. *Int. J. Mol. Sci.* **2020**, *21*, 8854, https://doi.org/10.3390/ijms21228854.
3. Iqbal, I.; Bajeli, S.; Akela, A.; Kumar, A. Bioenergetics of *Mycobacterium*: An Emerging Landscape for Drug Discovery. *Pathogens* **2018**, *7*, 24, https://doi.org/10.3390/pathogens7010024.

4. Beites, T.; O'Brien, K.; Tiwari, D.; Engelhart, C.A.; Walters, S.; Andrews, J.; Yang, H.-J.; Sutphen, M.L.; Weiner, D.M.; Dayao, E.K.; et al. Plasticity of the Mycobacterium tuberculosis respiratory chain and its impact on tuberculosis drug development. *Nat. Commun.* **2019**, *10*, 1–12, https://doi.org/10.1038/s41467-019-12956-2.

6.6. Challenges in the Synthesis of a Chiral Heterocyclic Compound

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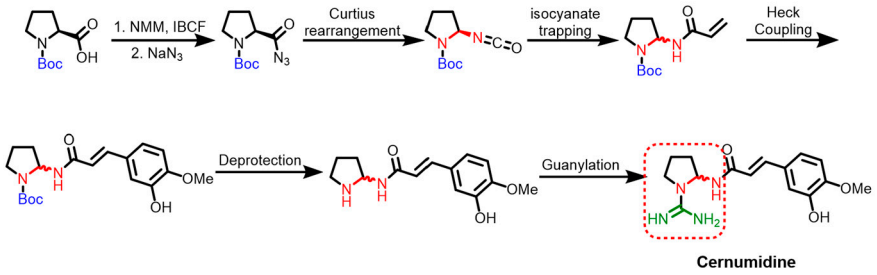
Cernumidine is an alkaloid of natural origin with great biological significance [1,2], but chemically, this molecule is equally interesting. Cernumidine has a unique structure with an aminoguanidine core and an asymmetric center, resulting in a challenging aminopyrrolidine nucleus. Our group developed a synthetic route to attain cernumidine (Scheme 1) along with derivatives having the Curtius rearrangement as the key step. The rearrangement allowed the formation of the aminal core and consequently allowed us to reach a family of aminopyrrolidine compounds through the formation of the amide group.



Scheme 1. Synthetic route designed to the synthesis of cernumidine and the aminopyrrolidine nucleus.

Surprisingly, racemization was observed on the Curtius rearrangement, which was not expected [3], allowing us to reach mixtures of enantiomers as confirmed by X-ray crystallography and optical rotation. Cernumidine was obtained with a slight enantiomeric excess.

In this work, we will present the intricacies found in this search for a chiral synthetic pathway.

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References

1. Lopes, L.C.; Roman, B.; Medeiros, M.A.; Mukhopadhyay, A.; Utrilla, P.; Galvez, J.; Mauriño, S.G.; Moltiva, V.; Lourenço, A.; Feliciano, A.S. Cernumidine and isocernumidine, new type of cyclic guanidine alkaloids from Solanum cernuum. *Tetrahedron Lett.* **2011**, *52*, 6392–6395, https://doi.org/10.1016/j.tetlet.2011.09.060.
2. Miranda, M.A.; Mondal, A.; Sachdeva, M.; Cabral, H.; Neto, Y.A.A.H.; Khan, I.; Groppo, M.; McChesney, J.D.; Bastos, J.K. Chemosensitizing Effect of Cernumidine Extracted from *Solanum cernuum* on Bladder Cancer Cells *in Vitro*. *Chem. Biodivers.* **2019**, *16*, e1900334, https://doi.org/10.1002/cbdv.201900334.

3. Ghosh, A.K.; Sarkar, A.; Brindisi, M. The Curtius rearrangement: mechanistic insight and recent applications in natural product syntheses. *Org. Biomol. Chem.* **2018**, *16*, 2006–2027, https://doi.org/10.1039/c8ob00138c.

6.7. Synthesis of Dicarboxymethyl Cellulose: A Green Metrics Perspective

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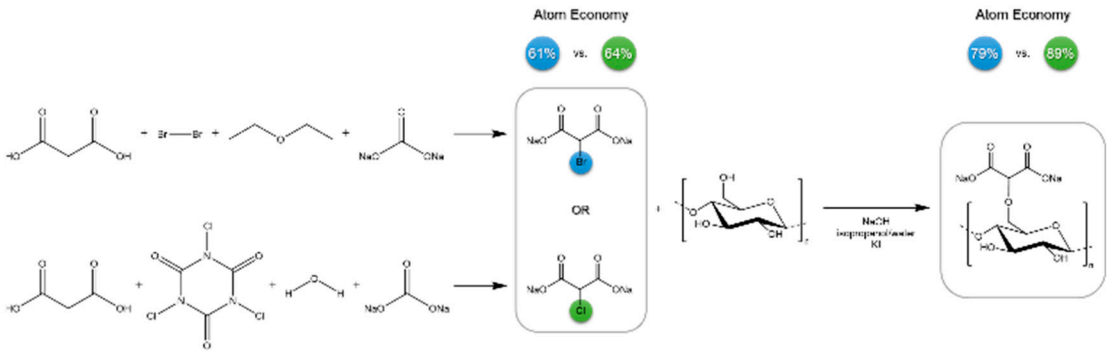
Cellulose is one of the most used polysaccharides due to its abundance and availability. Even though the use of cellulose as a starting material contributes to a greener process, the synthesis of cellulose derivatives is not always sustainable. Solvents, reactants, catalysts and cellulose modification methods play an important role in the environmental impact of the derivatization processes [1].

Dicarboxymethyl cellulose (DCMC) is a recent cellulose-based polymer produced by the heterogeneous etherification of cellulose with a β-halocarbonyl compound. DCMC has been produced with sodium bromo- and chloromalonate (Na-BMA and Na-CMA, respectively) [2,3]. Both the polymer and the etherifying agent's synthesis were evaluated under the scope of green chemistry.

Green metrics were used to determine the influence of the halogen-substituted carbonyl compound on the sustainability of this process. Atom economy and E-factor were calculated for the synthesis of the electrophiles and the respective polymer production. The reaction synthesis of Na-BMA and Na-CMA has a similar atom economy (61% and 64%, respectively). However, the preparation of DCMC with chlorocarbonyl has a significantly higher atom economy when compared with the bromocarbonyl (Scheme 1). Nevertheless, the environmental factor (E-factor) showed that the production of Na-BMA results in five times the amount of waste that Na-CMA produces (5.71 versus 0.99 kg waste/kg product), whereas the preparation of DCMC from both electrophiles produced 36 kg of waste per kg of product. Overall, the reactions including the chlorocarbonyl electrophiles are shown to have a higher atom economy while producing less waste. Therefore, DCMC produced with sodium chloromalonate is suggested to be the more sustainable option [4].



Scheme 1. Impact of the electrophile on the atom economy of the synthesis of dicarboxymethyl cellulose.

Funding: We thank the Fundação para a Ciência e Tecnologia for financial **support** through the PhD grant DFA/BD/5529/2020 and Associate Laboratory for Green Chemistry—

LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

References

1. Onwukamike, K.N.; Grelier, S.; Grau, E.; Cramail, H.; Meier, M.A.R. Critical Review on Sustainable Homogeneous Cellulose Modification: Why Renewability Is Not Enough. *ACS Sustain. Chem. Eng.* **2019**, *7*, 1826–1840, https://doi.org/10.1021/acssuschemeng.8b04990.
2. Chagas, R.; Gericke, M.; Ferreira, R.B.; Heinze, T.; Ferreira, L.M. Synthesis and characterization of dicarboxymethyl cellulose. *Cellulose* **2019**, *27*, 1965–1974, https://doi.org/10.1007/s10570-019-02952-6.
3. Gago, D.; Chagas, R.; Ferreira, L. The Effect of Dicarboxymethyl Cellulose on the Prevention of Protein Haze Formation on White Wine. *Beverages* **2021**, *7*, 57, https://doi.org/10.3390/beverages7030057.
4. Mulvihill, M.J.; Beach, E.S.; Zimmerman, J.B.; Anastas, P.T. Green Chemistry and Green Engineering: A Framework for Sustainable Technology Development. *Annu. Rev. Environ. Resour.* **2011**, *36*, 271–293, https://doi.org/10.1146/annurev-environ-032009-095500.

6.8. Laurus Azorica Leaves: Sesquiterpene Lactones and Antiaging Activity

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Plants are a relevant source of biologically active compounds for skin protection [1]. *Laurus azorica* (Seub.) Franco, an endemic species from Azores, was traditionally used as a disinfectant, and the oil from its berries was used to treat wounds [2,3]. This species is barely studied concerning its chemical constituents and biological activities.

In this study, three sesquiterpene lactones, costunolide (**1**), 11,13-dehydrosantonin (**2**) and reynosin (**3**) (Figure 1), were isolated for the first time on the hexane fraction of the ethanol extract from *Laurus azorica* leaves by chromatographic techniques. The chemical structure of the compounds was elucidated, using spectroscopic techniques, such as NMR 1D (^{1}H, ^{13}C, DEPT 90 e 135) and 2D (COSY, HSQC, HMBC e H2BC) and ESIMS. These compounds have already been isolated and identified in the species *Laurus nobilis*, and in *Laurus novocanariensis,* only costunolide and reynosin were identified [4,5]. The three sesquiterpene lactones have been described as having cytotoxic activity [4].

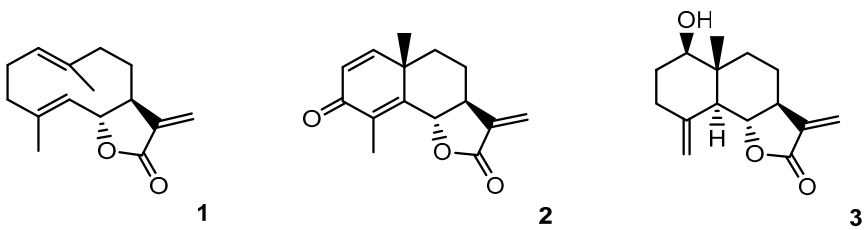

Figure 1. Chemical structure of compounds isolated from *Laurus azorica* leaves.

The in vitro antiaging activity was also evaluated. The ethanol extract exhibited an excellent antioxidant activity in ABTS and β-carotene bleaching assays (IC_{50} = 6.78 μg/mL and IC_{50} = 10.41 μg/mL, respectively) and moderate inhibition activity of tyrosinase

enzyme (IC_{50} = 12.04 μg/mL). In β-carotene bleaching assay, hexane fraction exhibited an IC_{50} = 14.74 μg/mL, which was comparable to the gallic acid used as standard (IC_{50} = 14.56 μg/mL), while costunolide was shown to be very active (IC_{50} = 4.08 μg/mL).

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References

1. Faccio, G. Plant Complexity and Cosmetic Innovation. *iScience* **2020**, *23*, 101358, https://doi.org/10.1016/j.isci.2020.101358.
2. Braga, T. *Plantas usadas na medicina popular*, 2nd ed.; Amigos dos Açores: Ponta Delgada, Portugal, 2006; p. 47.
3. Pontes, G.; Braga, T. *Plantas nativas dos Açores*; Amigos dos Açores: Ponta Delgada, Portugal, 2004; p. 32.
4. Barla, A.; Topçu, G.; Öksüz, S.; Tümen, G.; Kingston, D.G.I. Identification of cytotoxic sesquiterpenes from Laurus nobilis L. *Food Chem.* **2007**, *104*, 1478–1484.
5. Fraga, B.M.; Terrero, D.; Cabrera, I.; Reina, M. Studies on the sesquiterpene lactones from Laurus novocanariensis lead to the clarification of the structures of 1-epi-tatridin B and its epimer tatridin B. *Phytochemistry* **2018**, *153*, 48–52.

6.9. Dual Inhibition of Carbohydrate-Hydrolyzing Enzymes α-Amylase α-Glucosidase by Flavonoids

Carina Proença [1,*], Marisa Freitas [1], Ana T. Rufino [1], José Miguel P. F. Oliveira [1], Artur M. S. Silva [2], Pedro A. Fernandes [3] and Eduarda Fernandes [1,*]

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Type 2 diabetes (T2D) is characterized by the presence of insulin deficiency and/or resistance, leading to the progressive development of complications such as neuropathy, nephropathy, and retinopathy. According to the latest data released by the International Diabetes Federation, about 537 million adults are living with diabetes, and this disease is responsible for 6.7 million deaths in 2021 [1]. One class of antidiabetic agents currently available is the α-glucosidase inhibitors, which also inhibit α-amylase. Both enzymes are key carbohydrate hydrolases that regulate blood glucose levels by sequentially hydrolyzing starch to produce glucose. Therefore, the inhibition of α-glucosidase and α-amylase activity is a strategy to retard the absorption of glucose and reduce the postprandial hyperglycemia. Acarbose, voglibose and miglitol are clinically approved α-glucosidase inhibitors used for the management of T2D. These agents, as strong inhibitors of α-glucosidase and α-amylase, are however associated with frequent gastrointestinal adverse effects, including flatulence, diarrhea, and abdominal distention, which limit their clinical application. However, it was shown that only a mild inhibition of pancreatic α-amylase is required in order to avoid gastrointestinal side effects as a result of excessive bacterial fermentation of carbohydrates in colon. Based on this background, numerous efforts have been carried out to discover new and selective α-glucosidase inhibitors. Flavonoids are heterocyclic phenolic compounds

widely distributed in nature, with well-known biological activities, including antidiabetic properties by acting on different T2D targets [2–4].

The aim of the present work was to evaluate the inhibitory activity of a group of flavonoids on pancreatic α-amylase (porcine) and α-glucosidase (yeast and human (Caco-2/TC7 cells)). For this purpose, the enzyme-catalyzed hydrolysis of the substrate (selected according to each of the mentioned enzymes) was measured by monitoring the absorbance or fluorescence signal of the generated product.

The obtained results suggest that the presence of -OH groups at 3-position of C ring, at 3′- and 4′-positions of B ring, and at 7- and 8-positions of A ring is favorable for the simultaneous mild inhibition of α-amylase and stronger inhibition of α-glucosidase. Although more studies are needed, these data allowed the disclosure of the most important substituents in the flavonoid scaffold with potential to be used as an alternative option to the conventional α-glucosidase inhibitors used in T2D therapy [2–4].

Funding: This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020. This work also received financial support from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-029241) and national funds (FCT) through project PTDC/MED-QUI/29241/2017. ATR and CP thank the FCT for the funding through the project PTDC/MED-QUI/29243/2017. JMPFO thanks the FCT for funding through program DL 57/2016—Norma transitória (ref. SFRH/BPD/74868/2010). Marisa Freitas acknowledges her contract under the CEEC Individual (2020.04126.CEECIND/CP1596/CT0006).

References

1. IDF Diabetes Atlas, 2021. Available online: https://diabetesatlas.org/(accessed on 15 November 2021).
2. Proença, C.; Freitas, M.; Ribeiro, D.; Oliveira, E.F.T.; Sousa, J.L.C.; Tomé, S.M.; Ramos, M.J.; Silva, A.M.S.; Fernandes, P.A.; Fernandes, E. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure-activity relationship study. *J. Enzyme Inhib. Med. Chem.* **2017**, *32*, 1216–1228, https://doi.org/10.1080/14756366.2017.1368503.
3. Proença, C.; Freitas, M.; Ribeiro, D.; Tomé, S.M.; Oliveira, E.F.T.; Viegas, M.; Araújo, A.; Ramos, M.J.; Silva, A.M.S.; Fernandes, P.A.; Fernandes, E. Evaluation of a flavonoids library for inhibition of pancreatic α-amylase towards a structure-activity relationship. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 577–588, https://doi.org/10.1080/14756366.2018.1558221.
4. Proença, C.; Rufino, A.T.; Oliveira, J.M.P.F.; Freitas, M.; Fernandes, P.A.; Silva, A.M.S.; Fernandes, E. Inhibitory activity of flavonoids against human sucrase-isomaltase (α-glucosidase) activity in a Caco-2/TC7 cellular model. *Food Funct.* **2022**, *13*, 1108–1118, https://doi.org/10.1039/d1fo02995a.

7. Poster Presentations

7.1. Synthesis of Novel Hybrid Compounds to Obtain New Anticancer Agents with Dual Targeting p53

Ricardo Ferreira, Lídia M. Gonçalves, Valentina Barcherini, Ana P. Francisco and Maria M. M. Santos *

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p53 is a tumor suppressor protein and is responsible for the integrity of the cells, controlling cell cycle arrest and apoptosis. The mutation (mut) of p53 occurs in approximately 50% of human cancers, while in the remaining 50% of cases, wild-type (wt) p53 is inhibited by its main negative regulators (MDM2/MDMX). For this reason, there is a high interest in the reactivation of the p53 in order to exert its tumor suppressor function in tumor cells. In

the last few years, hybrid compounds emerged as an advantageous therapeutic approach in cancer therapy. In this approach, two distinct pharmacophores that independently act at two distinct pharmacological target structures are covalently connected by a linker in one unique molecule.

In the last few years, our research group has been involved in the development of novel p53 activators. In particular, we developed the enantiopure tryptophanol-derived oxazoloisoindolinone **1**, which inhibits the growth of wt p53- and mut p53R280K-expressing tumor cells by p53-dependent cell cycle arrest and/or apoptosis [1]. This compound also has the ability to reactivate other hotspot p53 mutations with high clinical relevance [2]. Moreover, we developed the spiropyrazoline oxindole **2**, which has antiproliferative activity in breast (MCF-7) and colon (HCT116) cancer cells [3]. This compound was shown to induce autophagy in ovarian cancer cells (A2780) and to interact with BSA with a Kb value of 3.14 $\times$ 10^6 M^{-1}, indicating that it can be efficiently transported by serum proteins in blood [4].

In this communication, we will present our studies on the design and synthesis of hybrid compounds combining, in one unique molecule, compounds **1** and **2** (Figure 1) in order to obtain novel anticancer agents.

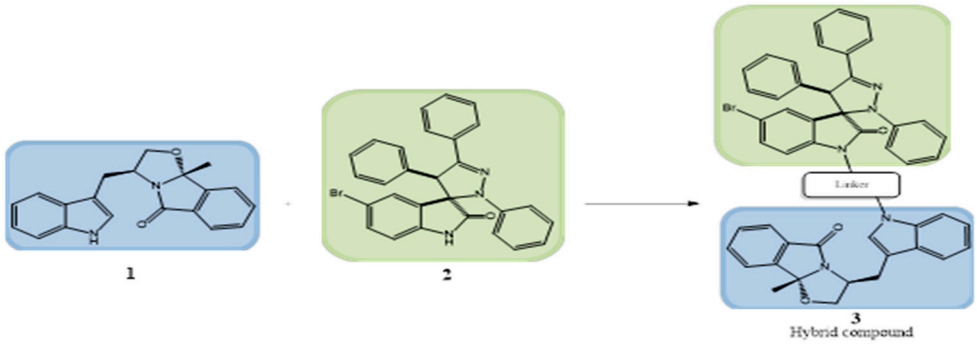

Figure 1. Synthetic approach for the synthesis of the hybrid compounds.

Funding: This work was financed by national funds from the FCT through iMed.ULisboa (UIDB/04138/2020), projects PTDC/QUIQOR/29664/2017 and PTDC/QUI-QOR/1304/2020 and PhD fellowship PD/BD/143126/2019 (V.B.).

References

1. Soares, J.; Raimundo, L.; Pereira, N.A.L.; Monteiro, Â.; Gomes, S.; Bessa, C.; Pereira, C.; Queiroz, G.; Bisio, A.; Fernandes, J.; et al. Reactivation of wild-type and mutant p53 by tryptophanolderived oxazoloisoindolinone SLMP53-1, a novel anticancer small-molecule. *Oncotarget* **2016**, *7*, 4326–4343, https://doi.org/10.18632/oncotarget.6775.
2. Gomes, A.S.; Ramos, H.; Gomes, S.; Loureiro, J.; Soares, J.; Barcherini, V.; Monti, P.; Fronza, G.; Oliveira, C.; Domingues, L.; et al. SLMP53-1 interacts with wild-type and mutant p53 DNA-binding domain and reactivates multiple hotspot mutations. *Biochim. Biophys. Acta (BBA) Gen. Subj.* **2020**, *1864*, 129440, https://doi.org/10.1016/j.bbagen.2019.129440.
3. Nunes, R.C.; Ribeiro, C.J.A.; Monteiro, Â.; Rodrigues, C.M.P.; Amaral, J.D.; Santos, M.M.M. In vitro targeting of colon cancer cells using spiropyrazoline oxindoles. *Eur. J. Med. Chem.* **2017**, *139*, 168–179, https://doi.org/10.1016/j.ejmech.2017.07.057.
4. Raposo, L.R.; Silva, A.; Silva, D.; Roma-Rodrigues, C.; Espadinha, M.; Baptista, P.V.; Santos, M.M.; Fernandes, A.R. Exploiting the antiproliferative potential of spiropyrazoline oxindoles in a human ovarian cancer cell line. *Bioorganic Med. Chem.* **2020**, *30*, 115880, https://doi.org/10.1016/j.bmc.2020.115880.

7.2. Monomethine Cyanine Dyes as Promising Anticancer Agents

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In today's world, cancer is regarded as one of the most prevalent diseases. Nowadays, several treatments are available for this disorder, among which the most usual are surgery, chemotherapy, radiotherapy, and photodynamic therapy (PDT). However, to innovate these therapies and improve efficacy/safety profiles, new compounds are being synthesized and tested. Cyanine dyes, discovered by Greville in 1856, have been widely studied from this point of view. As an example, squarylium cyanine dyes have been recently described as potential sensitizers for PDT, being typically non-cytotoxic in the dark [1–3]. Interestingly, cyanine dyes are cationic and tend to localize in the mitochondria of cancer cells with a greater selectivity over normal cells. In this context, despite being known for decades, to the best of our knowledge, cyanines have been very rarely explored as antiproliferative agents on their own. In this context, several symmetric and asymmetric cyanine dyes were tested by us with a focus on monomethine cyanines. For this, a 10 μM screening of representative twenty-five monomethine cyanine dyes (Figure 1) in three cancer cell lines and in normal human dermal fibroblasts (NHDF) was performed. Some of them have demonstrated excellent anticancer potential and selectivity over normal cells. The half-maximal inhibitory concentration (IC_{50}) of most active dyes was determined to evaluate their possible use as potential selective anticancer agents. An IC_{50} value of 0.01 μM for the Caco-2 cancer cell line and a selectivity index of 250 in relation to normal cells for the most active dye inspire additional future studies with a view in the potential applicability of this type of dye in cancer treatment.

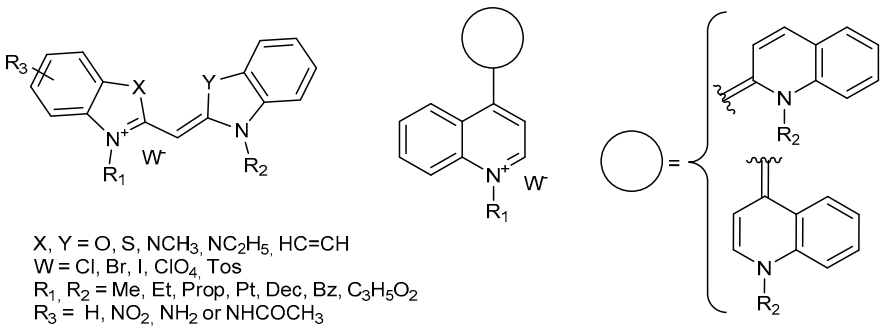

Figure 1. General structure of representative cyanine dyes under study.

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References

1. Lima, E.; Ferreira, O.; Gomes, V.S.D.; Santos, A.O.; Boto, R.E.; Fernandes, J.R.; Almeida, P.; Silvestre, S.M.; Reis, L.V. Synthesis and in vitro evaluation of the antitumoral phototherapeutic potential of squaraine cyanine dyes derived from indolenine. *Dyes Pigm.* **2019**, *167*, 98–108, https://doi.org/10.1016/j.dyepig.2019.04.007.

2. Lima, E.; Ferreira, O.; Silva, J.F.; Santos, A.O.; Boto, R.; Fernandes, J.R.A.; Almeida, P.; Silvestre, S.M.; Reis, L.V. Photodynamic activity of indolenine-based aminosquaraine cyanine dyes: Synthesis and in vitro photobiological evaluation. *Dye. Pigment.* **2019**, *174*, 108024, https://doi.org/10.1016/j.dyepig.2019.108024.
3. Fernandes, T.C.D.; Lima, E.; Boto, R.E.; Ferreira, D.; Fernandes, J.R.; Almeida, P.; Ferreira, L.F.V.; Silva, A.M.; Reis, L.V. In vitro phototherapeutic effects of indolenine-based mono- and dithiosquaraine cyanine dyes against Caco-2 and HepG2 human cancer cell lines. *Photodiagnosis Photodyn. Ther.* **2020**, *31*, 101844, https://doi.org/10.1016/j.pdpdt.2020.101844.

7.3. Bis-Thiobarbiturates with Xanthine Oxidase Inhibitory and Antiproliferative Activity

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Due to their wide range of biological activities, barbituric and thiobarbituric acid derivatives have attracted attention from the scientific community. In addition to the well-known sedative–hypnotic activities, recently, their interesting anticancer, antiviral, antifungal, antimicrobial and antihyperuricemic effects have been demonstrated. As a result of the overproduction and/or underexcretion of uric acid, hyperuricemia can lead to gout. Xanthine oxidase (XO), the enzyme that catalyzes the oxidative hydroxylation of hypoxanthine and xanthine to produce uric acid and reactive oxygen species (ROS), is the main target in hyperuricemia treatment. Allopurinol and febuxostat, the main drugs in the market acting by XO inhibition, have several adverse effects [1]. Thus, looking for new, alternative, and more potent XO inhibitors with less side effects, a series of about thirty bis-thiobarbiturates (Figure 1) was synthesized in moderate to excellent yields, and their capacity as xanthine oxidase inhibitors as well as free radical scavengers were evaluated. On the other hand, although there is no direct relationship between the use of XO inhibitors and a good prognosis in cancer treatment, the expression and activity of this enzyme have been negatively associated with a high degree of malignancy and a worse prognosis in some types of cancer, namely of the breast and gastrointestinal tract. Therefore, the antiproliferative potential of all bis-thiobarbiturates against colorectal adenocarcinoma Caco-2, breast cancer MCF-7 and non-tumoral NHDF cell lines was also tested. Overall, these molecules were most potent as XO inhibitors, determining half-maximal inhibitory concentrations below 1 μM for some compounds. Interestingly, as the most active bis-thiobarbiturates under study are nearly ten times more potent than the commercial drug allopurinol, the presented results were already the subject of a national patent [2].

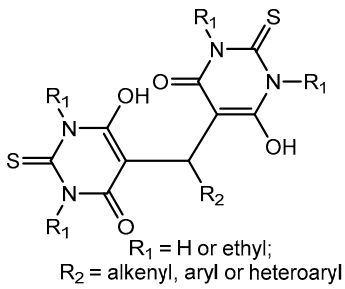

Figure 1. General structure of bis-thiobarbiturates under study.

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References

1. Serrano, J.L.; Figueiredo, J.; Almeida, P.; Silvestre, S. From Xanthine Oxidase Inhibition to in Vivo Hypouricemic Effect: An Integrated Overview of in Vitro and in Vivo Studies with Focus on Natural Molecules and Analogues, *Evid. Based Complementary Altern. Med.* **2020**, *2020*, 9531725, https://doi.org/10.1155/2020/9531725.
2. Serrano, J.L.; Lopes, D.; Reis, M.J.A.; Boto, R.E.F.; Silvestre, S.; Almeida, P. Bisthiobarbiturates as Promising Xanthine Oxidase Inhibitors: Synthesis and Biological Evaluation. *Biomedicines* **2021**, *9*, 1443. https://doi.org/10.3390/biomedicines9101443
3. Serrano, J.L.; Lopes, D.; Reis, M.J.A.; Boto, R.E.F.; Silvestre, S.; Almeida, P. Bispirimidinonas como inibidores da Xantina Oxidase para o tratamento de condições patológicas causadas por hiperuricemia. Patent PT116062, 20 January 2020.

7.4. Scale-Up Studies of the Synthesis of Phenylpropenoid Sucrose Ester

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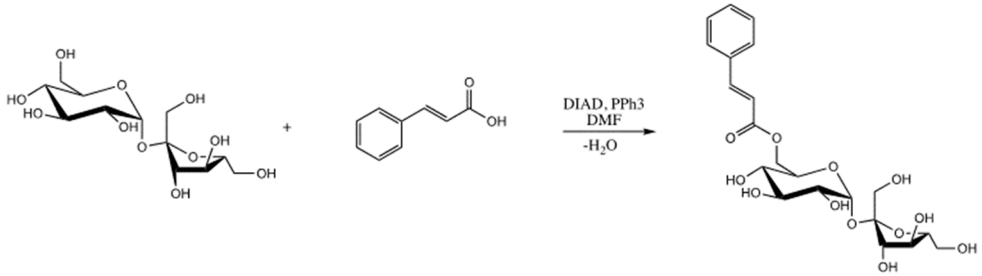
Phenylpropenoid sucrose esters are constituents of herbs such as *Scrophularia ningpoensis*, which have been used in traditional Chinese medicine and are known to have many therapeutic effects, including antiaging ones [1]. A market study over the antiaging cosmetics shows that the market has been growing at a steady 5% over the period from 2017 to 2020 and is valued at 38.62 billion USD in 2020. Until now, the only known method for obtaining the title substances was extraction from raw plant material, which is a very low yielding process (7.87×10^{-6}%) [2]. Using a selective esterification method such as Mitsunobu's reaction, we can synthesize the target compound with much higher yield [3], as shown in Scheme 1. The objective of this research is to evaluate whether it is possible to obtain the natural esters by synthesis on a larger scale (10 kg per batch) and if it will be profitable. In the scope of this work, we have performed several experiments—using a mole ratio of 1:2 between the sucrose, and other reagents yielded 23% conversion of sucrose on a one-gram scale. Next, we tried to optimize the usage of the more expensive reagents by changing the mole ratio to 1:1 but yielding much lower conversion. UV-VIS spectrometry is being studied for monitoring of the chromatography purification on a larger scale. The time to complete a batch is about 48 h, and the bottleneck of the process is the reaction time, which is at least 24 h. The next step will be a further scale-up of the process. Calculations of the dimensions, time, energy, and materials consumption of an industrial installation are being performed.



Scheme 1. Reaction scheme of the synthesis of 6-*O*-cinnamoyl sucrose.

Funding: This work was supported by the Associate Laboratory for Green Chemistry—LAQV which is financed by national funds from FCT/MCTES (UIDB/50006/2020 and UIDP/50006/2020).

References

1. Zhang, Y.-F.; Liu, L.-J.; Xu, F.; Shang, M.-Y.; Liu, G.-X.; Cai, S.-Q. Investigation of the In Vivo Metabolism of Sibirioside A and Angoroside C in Rats by HPLC-ESI-IT-TOF-MSn. *Molecules* **2018**, *23*, 2702, https://doi.org/10.3390/molecules23102702.
2. Li, Y.-M.; Jiang, S.-H.; Gao, W.-Y.; Zhu, D.-Y. Phenylpropanoid glycosides from Scrophularia ningpoensis. *Phytochemistry* **2000**, *54*, 923–925, https://doi.org/10.1016/s0031-9422(00)00171-0.
3. Petrova, K.T.; Ferreira, I.C.F.R.; Calhelha, R.M.; Sokovic, M. Phenylpropenoid saccharide esters, methods and uses thereof. Patent No. WO2020183442 A2, 16 March 2020.

7.5. Photodynamic Antitumor Effects Evaluation of a Picolylamine-Bearing Benz[e]indole-Based Squaraine Dye against HeLa Cells

Eurico Lima [1,2], Andreia G. Barroso [1], Octávio Ferreira [2], Renato E. Boto [2], José R. Fernandes [1], Paulo Almeida [2], Samuel M. Silvestre [2], Adriana O. Santos [2] and Lucinda V. Reis [1,*]

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Photodynamic therapy is an innovative treatment approach broadly directed toward oncological diseases [1]. Its applicability and efficiency are closely related to the interaction of three main components, namely a photosensitizer, light and molecular triplet oxygen, which should drive cell death [2]. Several studies have recently demonstrated that squaraine dyes, a class of squaric acid-derived organic dyes first synthesized in 1965, have a set of photophysical and photochemical properties that have made these compounds' potential photosensitizers for this therapeutic modality [3,4]. In the present work, the synthesis of a benz[e]indole picolylamine-bearing squaraine dye and the evaluation of its in vitro photoantiproliferative activity are presented. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was performed to assess the compounds' antiproliferative ability at several concentrations and irradiation conditions. Its cell location was determined by confocal microscopy, its genotoxicity was evaluated by comet assay, and studies to achieve the death mechanism and cell cycle were carried out by flow cytometry. Due to its extensive research use, HeLa cervical cancer cells were the chosen model for carrying out the tests. Overall, the dye showed significantly lower half-maximal inhibitory concentrations for irradiated than for dark conditions, evidencing photodynamic activity. No tumor selectivity was observed. The compound was shown to be located preferentially in the mitochondria, since its fluorescence colocalized with that emitted by Rhodamine 123, contrary to that used to label nucleic acids and lysosomes. The dye showed high genotoxicity, regardless of the irradiation condition. Given the cleavage of the genetic material observed by propidium iodide-staining flow cytometry, it could be concluded that the death mechanism related to the dye's photodynamic activity is probably via the apoptotic pathway. Non-significant effects were observed regarding its influence on the cell cycle.

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References

1. Allison, R.R.; Moghissi, K. Photodynamic Therapy (PDT): PDT Mechanisms. *Clin. Endosc.* **2013**, *46*, 24–29, https://doi.org/10.5946/ce.2013.46.1.24.
2. Agostinis, P.; Berg, K.; Cengel, K.A.; Foster, T.H.; Girotti, A.W.; Gollnick, S.O.; Hahn, S.M.; Hamblin, M.R.; Juzeniene, A.; Kessel, D.; et al. Photodynamic therapy of cancer: An update. *CA Cancer J. Clin.* **2011**, *61*, 250–281. https://doi.org/10.3322/caac.20114.
3. Lima, E.; Ferreira, O.; Silva, J.F.; Santos, A.O.; Boto, R.; Fernandes, J.R.A.; Almeida, P.; Silvestre, S.M.; Reis, L.V. Photodynamic activity of indolenine-based aminosquaraine cyanine dyes: Synthesis and in vitro photobiological evaluation. *Dye. Pigment.* **2019**, *174*, 108024, https://doi.org/10.1016/j.dyepig.2019.108024.
4. Friães, S.; Lima, E.; Boto, R.E.; Ferreira, D.; Fernandes, J.R.; Ferreira, L.F.V.; Silva, A.M.; Reis, L.V. Photophysicochemical Properties and In Vitro Phototherapeutic Effects of Iodoquinoline- and Benzothiazole-Derived Unsymmetrical Squaraine Cyanine Dyes. *Appl. Sci.* **2019**, *9*, 5414, https://doi.org/10.3390/app9245414.

7.6. Trans-A_2B-Corroles Containing a Hydrazone Moiety: A New Class of Photosensitizers for Photodynamic Therapy of Lung Cancer

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Lung cancer (LC) is the leading cause of cancer death worldwide. Photodynamic therapy (PDT) is a promising therapeutic option for LC that relies on photosensitizers (PS) that accumulate selectively in tumors and induce cytotoxicity upon irradiation via the generation of reactive oxygen species [1,2]. Our research group has been studying the reactivity of azoalkenes and nitrosoalkenes for the synthesis and functionalization of several heterocyclic systems [3]. Recently, we described an innovative synthesis of *trans*-A_2B-corroles containing an oxime moiety by exploring the chemistry of nitrosoalkenes toward dipyrromethanes [4]. In this communication, a novel approach to *trans*-A_2B-corroles bearing a hydrazone functional group based on the reactivity of azoalkenes is disclosed. The synthetic strategy involves the synthesis of bilanes via two consecutive hetero-Diels–Alder reactions or conjugated additions of in situ generated azoalkenes with dipyrromethanes, which are followed by oxidative macrocyclization (Figure 1). In addition, the potential of this new class of *trans*-A_2B-corroles as a PS for PDT of LC was evaluated. Firstly, the singlet quantum yield was determined, showing that all corroles have potential for application as PS. The in vitro results have shown that all corroles have high photocytotoxicity and low

or no dark-cytotoxicity, which supports their applicability as photosensitizers in PDT of lung cancer (Figure 1) [5].

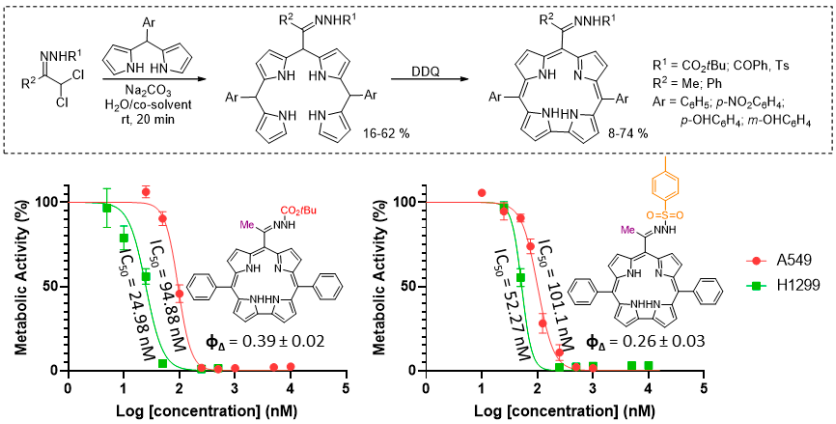

Figure 1. Synthesis of corroles and properties of most promising corroles.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249, https://doi.org/10.3322/caac.21660.
2. Allison, R.R.; Mota, H.C.; Sibata, C.H. Clinical PD/PDT in North America: An historical review. *Photodiagn. Photodyn. Ther.* **2004**, *1*, 263–277, https://doi.org/10.1016/s1572-1000(04)00084-5.
3. Lopes, S.M.M.; Cardoso, A.L.; Lemos, A.; Pinho e Melo, T.M.V.D. Recent Advances in the Chemistry of Conjugated Nitrosoalkenes and Azoalkenes. *Chem. Rev.* **2018**, *118*, 11324–11352, https://doi.org/10.1021/acs.chemrev.8b00375.
4. Lopes, S.M.M.; Pinho e Melo, T.M.V.D. Meso-Substituted Corroles from Nitrosoalkenes and Dipyrromethanes. *J. Org. Chem.* **2020**, *85*, 3328–3335, https://doi.org/10.1021/acs.joc.9b03151.
5. Costa, B. Corróis e hidrazonas: dois aliados na luta contra o cancro do pulmão. Master Thesis, University of Coimbra, Coimbra, Portugal, 2021.

7.7. Corrole Dimers as Photosensitizers: Synthesis and Antimicrobial Activity Studies

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Corrole oligomers have emerged as a new class of π-conjugated materials with unique functions allowing to modulate their optical, electrical, and magnetic properties [1]. Singly

linked (e.g., **1** and **2**, Figure 1) and doubly linked (e.g., **3**, Figure 1) corrole dimers have been synthesized via a multistep process that relied on regiospecific Pd-catalyzed oxidative-coupling reactions or by thermo-oxidative conditions [2–4]. The electronic spectra of corrole dimers such as **3** fall within the photodynamic therapy (PDT) therapeutic window (600–800 nm), which makes them promising candidates to be used as photosensitizers (PS) [3].

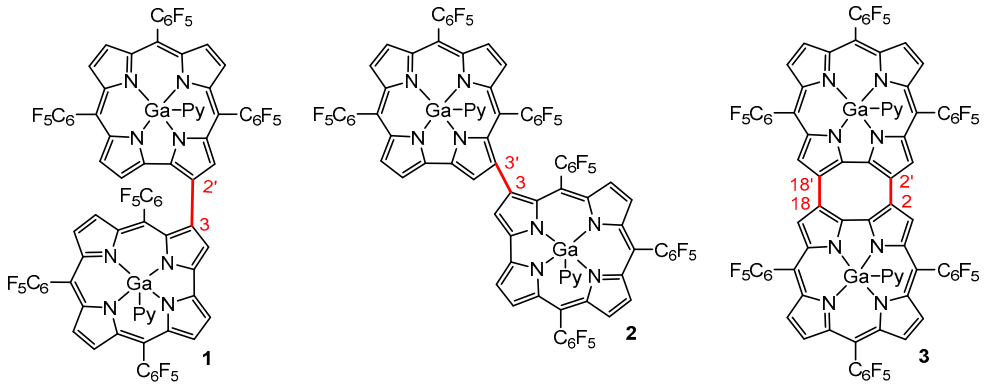

Figure 1. Chemical structure of singly (**1** and **2**) and doubly linked (**3**) corrole dimers.

The use of corrole oligomers as PS has not yet been explored. Following our interest in the development of new corrole PS to be used in antimicrobial photodynamic therapy (aPDT) [5], in this communication, we will discuss the potential of corrole dimers to photoinactivate a multiresistant *Staphylococcus aureus* strain. In addition, a peculiar alternative to the synthesis of dimers **1–3** mediated by acidic conditions, as well as their structural, photophysical, and photochemical characterization will be discussed.

Funding: Thanks are due to the University of Aveiro and FCT/MCTES for the financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020) and LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network, which partially support the NMR spectrometers by Infrastructure Project N° 022161 (co-financed by FEDER through COMPETE 2020, POCI and PORL and FCT through PIDDAC). This work was financially supported by the project Corlutna (POCI-01-0145-FEDER-031523 PTDC/QUI-ORG/31523/2017) funded by FEDER, through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. P.S.S.L (research contract) and M.B. (research grant BI/UI88/7701/2021) were also funded by project Corlutna (POCI-01-0145-FEDER-031523-PTDC/QUI-ORG/31523/2017).

References

1. Ooi, S.; Ueta, K.; Tanaka, T.; Osuka, A. Singly, Doubly, and Triply Linked Corrole Oligomers: Synthesis, Structures, and Linking Position Dependent Properties. *Chempluschem* **2019**, *84*, 578–588, https://doi.org/10.1002/cplu.201800570.
2. Hiroto, S.; Furukawa, K.; Shinokubo, H.; Osuka, A. Synthesis and biradicaloid character of doubly linked corrole dimers. *J. Am. Chem. Soc.* **2006**, *128*, 12380–12381. https://doi.org/10.1021/ja062654z.
3. Barata, J.F.B.; Silva, A.M.G.; Neves, M.G.P.M.S.; Tomé, A.C.; Silva, A.M.S.; Cavaleiro, J.A.S. β,β′-Corrole dimers. *Tetrahedron Lett.* **2006**, *47*, 8171–8174. https://doi.org/10.1016/j.tetlet.2006.09.026.
4. Bhowmik, S.; Kosa, M.; Mizrahi, A.; Fridman, N.; Saphier, M.; Stanger, A.; Gross, Z. The planar cyclooctatetraene bridge in bis-metallic macrocycles: isolating or conjugating? *Inorg. Chem.* **2017**, *56*, 2287–2296. https://doi.org/10.1021/acs.inorgchem.6b02944.
5. Cardote, T.A.F.; Barata, J.F.B.; Amador, C.; Alves, E.; Neves, M.G.P.M.S.; Cavaleiro, J.A.S.; Cunha, Â.; Almeida, A.; Faustino, M.A.F. Evaluation of meso-substituted

cationic corroles as potential antibacterial agents. *An. Acad. Bras. Cienc.* **2018**, *90*, 1175–1185. https://doi.org/10.1590/0001-3765201820170824.

7.8. New Insights on the Inhibition of Cyclooxygenases by 4-Styrylpyrazoles: An Enzymatic and Human Whole Blood Study

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Cyclooxygenases (COXs) catalyze the formation of prostaglandins, which are important mediators of inflammation, pain, cardiovascular disease, and cancer. COXs enzymes are mainly classified into two distinct isoenzymes, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is the constitutive isoform, and it is widely expressed in diverse tissues. It has a "housekeeping" role and is principally involved in tissue homeostasis [1]. COX-2 is a predominantly inducible enzyme that is rapidly expressed in response to several factors, including pro-inflammatory molecules. Despite the relevance in finding COX-2 selective inhibitors to modulate the inflammatory process, without gastric side effects, it is known that their long-term use is frequently associated to cardiac adverse effects [2]. Therefore, it is essential to find new and safer inhibitors of COXs enzymes, clarifying their selectivity. Celecoxib, a pyrazole derivative, was the first COX-2 selective inhibitor introduced in the clinic [3]. Therefore, we propose the study of the inhibitory activity of six 4-styrylpyrazoles, holding styryl groups with chloro, methoxy, trifluoromethyl and nitro substituents, against human COX-2, through a fluorometric, non-cellular, microanalysis screening system. These compounds were also studied ex vivo in human whole blood for their inhibitory activity against COX-1 and COX-2. Celecoxib and indomethacin were used as positive controls.

The obtained results from the microanalysis screening system showed that 4-styrylpyrazoles were able to inhibit COX-2, with IC_{50} values ranging from 23.0 $\pm$ 2.7 to 62.2 $\pm$ 3.7 μM. However, the most active compounds found in the in vitro non-cellular system were not the most effective in human blood, showing that protein-rich environments could lower their free concentrations, hindering their inhibitory effect. These results prompt us to refine the structures here proposed to find more effective compounds in a complex and physiological matrix, as human blood.

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References

1. Attiq, A.; Jalil, J.; Husain, K.; Ahmad, W. Raging the War Against Inflammation with Natural Products. *Front. Pharmacol.* **2018**, *9*, https://doi.org/10.3389/fphar.2018.00976.
2. Mahesh, G.; Anil Kumar, K.; Reddanna, P. Overview on the Discovery and Development of Anti-Inflammatory Drugs: Should the Focus Be on Synthesis or Degradation of PGE(2)? *J. Inflamm. Res.* **2021**, *14*, 253–263, https://doi.org/10.2147/jir.S278514.
3. Ansari, A.; Ali, A.; Asif, M.; Shamsuzzaman. Review: biologically active pyrazole derivatives. *New J. Chem.* **2017**, *41*, 16–41, https://doi.org/10.1039/C6NJ03181A.

7.9. Styrylpyrazoles and Chromone Derivatives as Possible Glycogen Phosphorylase Inhibitors: A Combined Enzyme Kinetic and Molecular Docking Study

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The liver plays a major role in the maintenance of normal glucose homeostasis. However, the net hepatic glucose metabolism is dysregulated in type 2 diabetes *mellitus* (DM). In fact, excessive hepatic glucose production is a major contributor to the rise of hyperglycemia. Considering that glycogen phosphorylase (GP) is a key enzyme in the glycogenolysis pathway, its inhibition constitutes a potential therapeutic target for type 2 DM management [1,2].

The antidiabetic activity of pyrazoles and chromone derivatives has been described, but whether GP inhibition contributes to this effect is still unknown. Thus, the aim of the present study was to evaluate the inhibitory activity of a panel of 52 structurally related compounds, including 4- and 5-styrylpyrazoles, flavonoids, 2-styrylchromones and 2-(4-arylbuta-1,3-dien-1-yl)chromones against GP activity, using a microanalysis screening system [3]. Molecular docking calculations and analyses were also performed to support the in vitro experimental findings.

The results showed that styrylpyrazoles were not able to inhibit GP activity. However, the chromone derivatives (flavonoids, 2-styrylchromones and 2-(4-arylbuta-1,3-dien-1-yl)chromones) revealed interesting inhibitory effects. The structure–activity relationship analysis showed that hydroxylations at the A and B rings on 2-styrylchromones and 2-(4-arylbuta-1,3-dien-1-yl)chromones, and hydroxylation of the A ring on flavonoids, were crucial for the inhibitory activity. Molecular docking analyses of 2-styrylchromones and 2-(4-arylbuta-1,3-dien-1-yl)chromones docking poses at the GP inhibitor binding site disclose that the active compounds of this family should have a characteristic binding pattern, requiring H-bond interactions with the two extreme ends of the pocket (ASN282/LYS289 and GLU382) to display inhibitory activity. The docking results for the flavonoid family show that the establishment of four simultaneous H-bonds, two on either "wall" of the pocket, drives activity. This is evident in the flavonoid norwogonin (5,7,8-trihydroxyflavone), which is one of the most active in this family and the only one where this effect is observed. In addition, the presence of high levels of glucose increased the inhibitory effect of the most effective compounds. This outcome could reduce the risk of hypoglycemia, which is a commonly reported side effect of antidiabetic agents.

In conclusion, this work gathers important considerations and provides a better understanding of novel potential scaffolds to the study of novel GP inhibitors.

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References

1. Petersen, M.C.; Vatner, D.F.; Shulman, G.I. Regulation of hepatic glucose metabolism in health and disease. *Nat. Rev. Endocrinol.* **2017**, *13*, 572–587, https://doi.org/10.1038/nrendo.2017.80.
2. Sharabi, K.; Tavares, C.D.J.; Rines, A.K.; Puigserver, P. Molecular pathophysiology of hepatic glucose production. *Mol. Asp. Med.* **2015**, *46*, 21–33, https://doi.org/10.1016/j.mam.2015.09.003.
3. Rocha, S.; Lucas, M.; Araújo, A.N.; Corvo, M.L.; Fernandes, E.; Freitas, M. Optimization and Validation of an In Vitro Standardized Glycogen Phosphorylase Activity Assay. *Molecules* **2021**, *26*, https://doi.org/10.3390/molecules26154635.

7.10. Reactivity of Ethyl Nitrosoacrylate toward Pyrrole, Indole and Pyrrolo[3,2-c]carbazole: An Experimental and Theoretical Study

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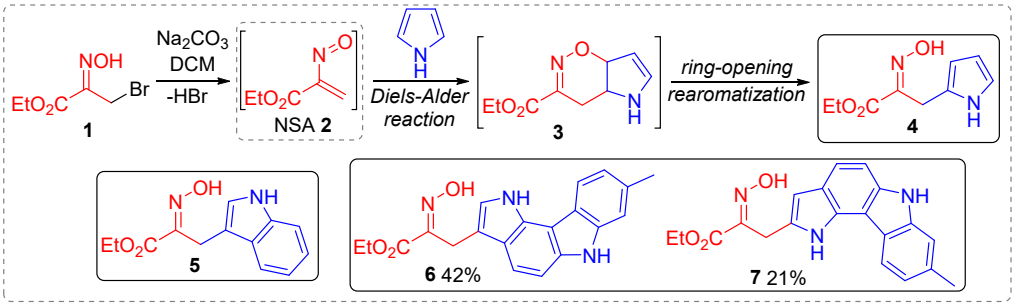
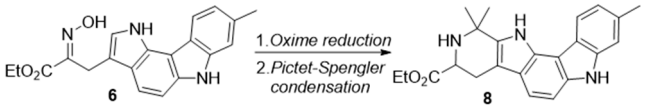
The study of nitrosoalkenes has shown that they are valued in organic synthesis as intermediates in the synthesis of a wide range of heterocyclic systems [1]. The pioneer work of Gilchrist and co-workers [2] showed that conjugated ethyl nitrosoacrylate (NSA **2**), generated in situ from the α-halo-oxime **1**, reacts with pyrrole, affording 2-alkylated pyrrole **4** as the only product through hetero-Diels–Alder (HDA) reaction [2,3]. On the other hand, indole undergoes alkylation at the 3 position on reacting with NSA **2**, giving the open chain oxime **5** via HDA reaction with the opposite regiochemistry [3,4]. Interestingly, the reaction of NSA **2** with 8-methyl-pyrrolo[3,2-c]carbazole, a tetracyclic ring system containing a pyrrole ring fused to the carbazole unit [5], gave two regioisomeric products (e.g., pyrrolo[3,2-c]carbazoles **6** and **7**) (Scheme 1). In order to investigate the observed and diverse regiochemistries, quantum chemical calculations, at the DFT level of theory, were carried out for the HDA of NSA **2** with pyrrole, indole and 8-methyl-pyrrolo[3,2-c]carbazole. For each heterocycle, relative stabilities of the different transition states involved in HDA reactions were calculated, considering both regiochemistries and both endo and exo approaches. These studies confirmed the observed regiochemistry. Furthermore, relative energy values of the HOMO and LUMO orbitals for the reactants were also calculated, corroborating that these heterocycles participate in inverse electron demand HDA reaction with nitrosoalkenes.



Scheme 1. Reactivity of nitrosoalkene **2** toward pyrrole, indole and 8-methyl-pyrrolo[3,2-*c*]carbazole.

Moreover, the reactivity of the new 3-alkylated pyrrolo[3,2-c]carbazole **6** was explored, leading to the construction of the hexahydropyrido[4′,3′:4,5]pyrrolo[3,2-c]carbazole system **8** (Scheme 2), whose structure was unambiguously established by X-ray crystallography (Figure 1).



Scheme 2. Synthesis of the hexahydropyrido[4′,3′:4,5]pyrrolo[3,2-*c*]carbazole **8**.

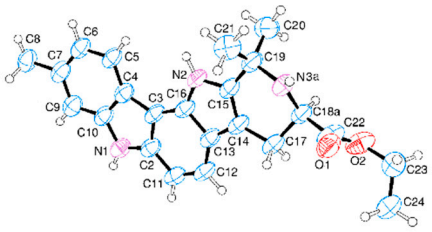


Figure 1. Crystal structure of **8**.

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References

1. Lopes, S.M.M.L; Cardoso, A.L.; Lemos, A.; Pinho e Melo, T. M. V. D. Recent Advances in the Chemistry of Conjugated Nitrosoalkenes and Azoalkenes. *Chem. Rev.* **2018**, *118*, 11324–11352.
2. Gilchrist, G.L.; Lemos, A. Reaction of Pyrroles with Ethyl 2-Nitroso- and 2-Azopropenoates, and with Ethyl Cyanoformate N-oxide: a Comparison of the Reaction Pathways. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1391–1395.
3. Nunes, S.C.C.; Lopes, S.M.M.; Gomes, C.S.B.; Lemos, A.; Pais, A.A.C.C.; Pinho e Melo, T.M.V.D. Reactions of Nitrosoalkenes with Dipyrromethanes and Pyrroles: Insight into the Mechanistic Pathway. *J. Org. Chem.* **2014**, *79*, 10456–10465.
4. Gilchrist, T.L.; Roberts, T. G. Addition and Cycloaddition Reactions of the Electrophilic Vinyl Nitroso Compounds 3-Nitrosobut-3-en-2-one, 2-Nitrosopropenal, and Ethyl 2-nitrosopropenoate. *J. Chem. Soc. Perkin Trans. 1* **1983**, 1283–1292.
5. Panice, M.R.; Lopes, S.M.M.; Sarragiotto, M.H.; Pinho e Melo, T.M.V.D. et. Al. New 3-Tetrazolyl-β-carbolines and β-Carboline-3-carboxylates with Anti-cancer Activity. *Eur. J. Med. Chem.* **2019**, *179*, 123–132.

6. Benzi, A.; Bianchi, L.; Maccagno, M.; Pagano, A.; Petrillo, G.; Tavani, C. Sequential Annulations to Interesting Novel Pyrrolo[3,2-c]carbazoles. *Molecules* **2019**, *24*, 3802.

7.11. trans-A_2B-Corroles Containing an Oxime Moiety: Novel Photosensitizers for Photodynamic Therapy of Lung Cancer

João Braz [1,*], Bruna D. P. Costa [1], Susana M. M. Lopes [1], Mafalda Laranjo [2], Marta Pineiro [1], Maria F. Botelho [2] and Teresa M. V. D. Pinho e Melo [1]

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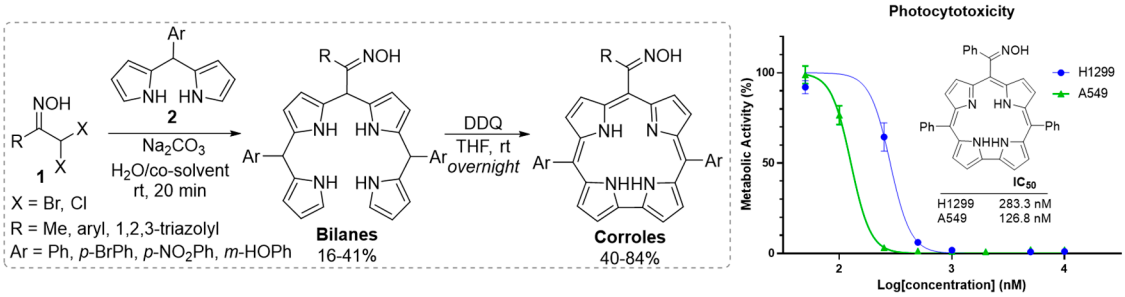
Photodynamic therapy (PDT) consists of a light-activated chemical reaction used to selectively destroy tissues through the generation of singlet oxygen and other reactive oxygen species (ROS). PDT is used as treatment to early-stage lung cancer and multifocal primary tumors, as a palliation strategy in patients with advanced disease and as an effective surgical adjuvant in patients with non-small cell lung cancer with pleural spread [1]. The application of this innovative therapy is highly dependent on an effective and selective photosensitizer (PS). In recent years, corroles have emerged as potential PSs for PDT [2]. In this context, we developed an unprecedent synthetic strategy to *trans*-A_2B-corroles containing an oxime moiety by exploring the reactivity of nitrosoalkenes [3]. The reaction of nitrosoalkenes, generated in situ from α,α-halo-oximes **1**, in the presence of dipyrromethanes **2** gave bilanes, which underwent oxidative macrocyclizations affording the target corroles in high yields (Scheme 1). In vitro assays carried out in lung cancer cell lines (H1299 and A549) showed that the synthesized corroles exhibit high photocytotoxicity with IC_{50} values in the nanomolar range. Moreover, none of the studied corroles showed dark cytotoxicity in both cell lines.



Scheme 1. Synthesis of *trans*-A_2B-corroles containing an oxime moiety showing low IC_{50} values in the nanomolar range against lung cancer cell lines.

Funding: The Coimbra Chemistry Centre (CQC) and Centre for Innovative Biomedicine and Biotechnology (CIBB) supported by the Portuguese Agency for Scientific Research (FCT) through projects UIDB/00313/2020, UIDP/QUI/00313/2020 and PTDC/QUI-QOR/0103/2021 (CQC), UIDB/04539/2020 and UIDP/04539/2020 (CIBB). João Braz thanks FCT/CQC for the PhD scholarship UI/BD/150880/2021. We also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Shafirstein, G.; Battoo, A.; Harris, K.; Baumann, H.; Gollnick, S.O.; Lindenmann, J.; Nwogu, C.E. Photodynamic Therapy of Non-Small Cell Lung Cancer. Narrative Review and Future Directions. *Ann. Am. Thorac. Soc.* **2016**, *13*, 265–275, https://doi.org/10.1513/AnnalsATS.201509-650FR.
2. Lopes, S.M.M.; Pinheiro, M.; Pinho e Melo, T.M.V.D. Corroles and Hexaphyrins: Synthesis and Application in Cancer Photodynamic Therapy. *Molecules* **2020**, *25*, 3450–3489.
3. Lopes, S.M.M.; Pinho e Melo, T.M.V.D. Meso-Substituted Corroles from Nitrosoalkenes and Dipyrromethanes. *J. Org. Chem.* **2020**, *85*, 3328–3335.

7.12. Exploring the Reactivity of Tetrazolyl-2H-Azirines toward Arynes: Selective Synthesis of Indole Derivatives

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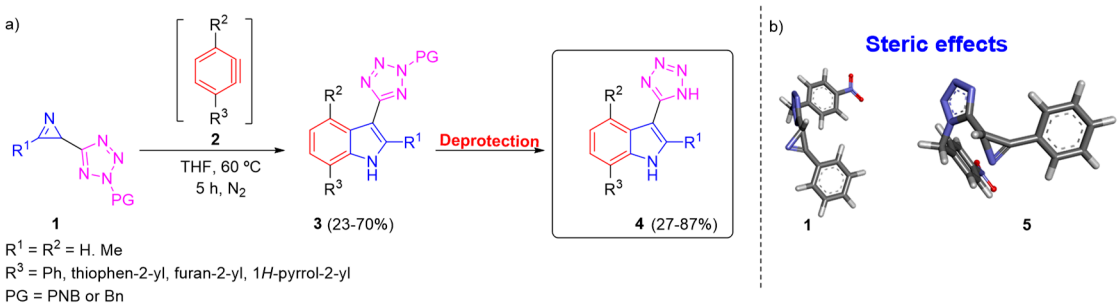
Indole is one of the most important pharmacophores found in several natural and synthetic compounds with diverse biological activities [1], which has led to the development of diverse indole synthetic routes. The chemistry of arynes has been one of our research interests [2,3] as well as the synthesis and reactivity of 2H-azirines [4–6]. Therefore, we decided to explore the reactivity of tetrazolyl-2H-azirines toward arynes in order to obtain a new class of indole derivatives bearing a tetrazole moiety (Scheme 1). Under the optimized reaction conditions, tetrazolyl-2H-azirine 1 (R1 = Ph, PG = PNB) reacted with aryne 2 (R2 = R3 = H), generated in situ from 2-(trimethylsilyl)aryl triflates, giving rise to indole 3 (R2 = R3 = H, R4 = Ph, PG = PNB) in a regioselective fashion in 70% yield. Unexpectedly, starting from 2H-azirines 5 having a 2-(4-nitrobenzyl)-1H-tetrazol-5-yl substituent, no reaction was observed. Computational studies were carried out allowing to rationalize the lack of reactivity of this 2H-azirine derivative. The synthetic methodology was extended to the synthesis of N-unsubstituted 3-tetrazolyl-indoles 3 bearing heteroaromatic substituents in moderate to good yields. Additionally, the symmetrical aryne (R2 = R3 = Me) generated from the corresponding precursor was also tested, leading to the target molecule in moderate to good yields. Finally, deprotection studies of the tetrazole moiety of indole derivatives 3 were carried out, allowing the synthesis of 3-tetrazolyl-indoles 4 in moderate to excellent yields. Further details of this study were discussed.



Scheme 1. (**a**) One-pot approach to 3-tetrazolyl-indoles from 2-(tetrazol-5-yl)-2H-azirines and arynes; (**b**) Computational rationalizations.

Funding: The Coimbra Chemistry Centre (CQC) is supported by the Portuguese Agency for Scientific Research, "Fundação para a Ciência e a Tecnologia" (FCT) through project UIDB/00313/2020 and UIDP/QUI/00313/2020, co-funded by COMPETE2020-UE. Carla Grosso thanks the FCT for the PhD fellowship (SFRH/BD/130198/2017). The authors also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Thanikachalam, P.V.; Maurya, R.K.; Garg, V. An insight into the medicinal perspective of synthetic analogs of indole: A review. *Eur. J. Med. Chem.* **2019**, *180*, 562–612, https://doi.org/10.1016/j.ejmech.2019.07.019.
2. Rocha Gonsalves, A.M.d'A.; Pinho e Melo, T.M.D.V. Synthesis of isoquinolines by cycloaddition of arynes to 1,2,4-triazines. *Tetrahedron* **1992**, *48*, 6821–6826, https://doi.org/10.1016/s0040-4020(01)89873-2.
3. Soares, M.I.L.; Nunes, C.M.; Gomes, C.S.; Pinho e Melo, T.M.V.D. Thiazolo[3,4-b]indazole-2,2-dioxides as Masked Extended Dipoles: Pericyclic Reactions of Benzodiazafulvenium Methides. *J. Org. Chem.* **2013**, *78*, 628–637, https://doi.org/10.1021/jo302463q.
4. Cardoso, A.L.; Gimeno, L.; Lemos, A.; Palacios, F.; Pinho e Melo, T.M.V.D. Té Neber Approach to 2-(Tetrazol-5-yl)-2H-Azirines. *J. Org. Chem.* **2013**, *78*, 6983–6991, https://doi.org/10.1021/jo4006552
5. Cardoso, A.L.; Lemos, A.; Pinho e Melo, T.M.V.D. Selective Synthesis of Tetrasubstituted 4-(Tetrazol-5-yl)-1H-imidazoles from 2-(Tetrazol-5-yl)-2H-azirines *Eur. J. Org. Chem.* **2014**, 5159–5165, HTTPS://DOI.ORG/10.1002/ejoc.201402559
6. Alves, C.; Grosso, C.; Barrulas, P.; Paixão, J.A.; Cardoso, A.L.; Burke, A.J.; Lemos, A.; Pinho e Melo, T.M.V.D. Asymmetric Neber Reaction in the Synthesis of Chiral 2-(Tetrazol-5-yl)-2H-Azirines *Synlett* **2020**, *31*, 553–558, HTTPS://DOI.ORG/10.1055/s-0039-1691533.

7.13. Application of epi-Cinchona Alkaloid Derivatives as Immobilized OrganoCatalysts in Solid and Liquid Phases

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Cinchona alkaloids are very well known in all the fields of Chemistry dealing with chirality, being recognized as the most powerful class of compounds in the realm of asymmetric organocatalysis during the last two decades [1–4].

In this work, we wanted to test a few functionalized epi-cinchona alkaloids as immobilized organocatalysts—given the advantage of easy recyclability enabling the reuse of the catalyst—in well-known benchmark reactions, verifying three aspects: (1) yield of the reaction, (2) enantioselectivity and (3) number of cycles where the catalyst retains its reactivity (Scheme 1).

Scheme 1. Strategy used to prepare and test our immobilized organocatalysts.

Two kinds of immobilizations were studied. In the first one, a new kind of solid support was used based on modified Controlled Porous Glass Beads (CPGs) and HybCPGs, named EziGTM (EziG Opal, EziG Coral and EziG Amber) from EnginZyme (www.enginzyme.com, accessed on 19 August 2022) [5]. In the second immobilization, deep nabling solvents (DESs) based on betaine were used as liquid support, enabling the recovery and reuse of the catalyst.

Both approaches gave good yields; very high enantioselectivity and a number of catalytic cycles could be achieved, as discussed in this presentation.

Funding: We thank the FCT for funding to LAQV-REQUIMTE through project UIDB/50006/2020.

References

1. *Organocatalysis: Stereoselective Reactions and Applications in Organic Synthesis*; Benaglia, M., Eds.; De Gruyter: Berlin, Germany, 2021.
2. Parvez, M.M.; Haraguchi, N.; Itsuno, S. Synthesis of Cinchona Alkaloid-Derived Chiral Polymers by Mizoroki–Heck Polymerization and Their Application to Asymmetric Catalysis. *Macromolecules* **2014**, *47*, 1922–1928, https://doi.org/10.1021/ma5001018.
3. Singh, G.; Yeboah, E. Recent applications of Cinchona alkaloid-based catalysts in asymmetric addition reactions. *Rep. Org. Chem.* **2016**, *6*, 47–75.
4. Marcelli, T. Organocatalysis: Cinchona catalysts. *WIRES Comput. Mol. Sci.* **2011**, *1*, 142–152, https://doi.org/10.1002/wcms.2.
5. Cassimjee, K.E.; Kadow, M.; Wikmark, Y.; Humble, M.S.; Rothstein, M.L.; Rothstein, D.M.; Bäckvall, J.-E. A general protein purification and immobilization method on controlled porosity glass: biocatalytic applications. *Chem. Comm.* **2014**, *50*, 9134–9137.

7.14. Synthesis of Unnatural Amino Acids by Cross-Dehydrogenative Coupling

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Metal-catalyzed cross-dehydrogenative coupling (CDC) has emerged in recent years as a powerful technique to make C–C bonds or C–X bonds (X = N, O, S, P) directly from two C–H bonds or a C–H and an X–H bond [1–3]. No prefunctionalization is required—only an oxidant to act as the terminal acceptor of the two hydrogen atoms. Coupled with homogeneous catalysis with earth-abundant metals, e.g., Cu, Fe or Co, cheap and nontoxic, CDC provides environmentally friendly processes which are atom-, energy-, time- and cost-efficient. We have explored this chemistry for the synthesis of modified, constrained amino acids [4–7]. Using imides as nucleophiles, a range of novel molecules (Figure 1 **3**) was obtained. Since the imide group is also an important pharmacophore present in a large range of medicinally important molecules, e.g., antiepileptic, antianxiety, antineoplastic and antipsychotic drugs, amongst others, the new compounds are of interest for peptidomimetics, drug design and synthetic applications [8].

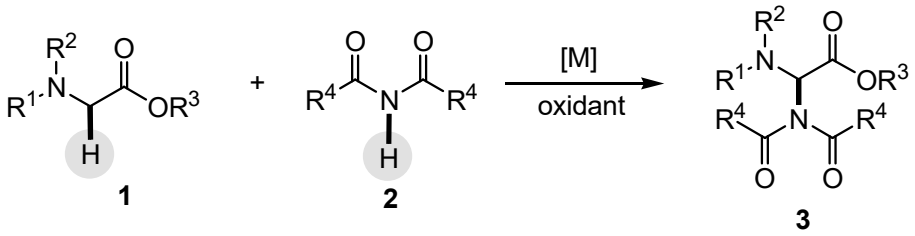

Figure 1. Unnatural amino acids synthesis by cross-dehydrogenative coupling.

Funding: We thank the financial support from the Fundação para a Ciência e a Tecnologia (FCT), Portugal, in the form of project UIDB/00100/2020 of Centro de Química Estrutural.

References

1. Faisca Phillips, A.M.; Pombeiro, A.J.L. Recent developments in transition metal-catalyzed cross-dehydrogenative coupling reactions of ethers and thioethers. *ChemCatChem* **2018**, *10*, 3354–3383, https://doi.org/10.1002/cctc.201800582.
2. Huang, C.-Y.; Kang, H.; Li, J.; Li, C.-J. En Route to Intermolecular Cross-Dehydrogenative Coupling Reactions. *J. Org. Chem.* **2019**, *84*, 12705–12721, https://doi.org/10.1021/acs.joc.9b01704.
3. Faisca Phillips, A.M.; C. Guedes da Silva, M.d.F.; Pombeiro, A.J.L. New Trends in Enantioselective Cross-Dehydrogenative Coupling. *Catalysts* **2020**, *10*, 529. https://doi.org/10.3390/catal10050529.
4. Faisca Phillips, A.M.; Pombeiro, A.J.L. Synthesis of Unnatural Amino Acids by Cross-Dehydrogenative Coupling. *Org. Biomol. Chem.* (*submitted*)
5. Blaskovich, M.A.T. Unusual Amino Acids in Medicinal Chemistry. *J. Med. Chem.* **2016**, *59*, 10807–10836, https://doi.org/10.1021/acs.jmedchem.6b00319.
6. Ramana, V.D.; Chandrasekharam, M. Cu(I)-Catalyzed amidation/imidation of N-arylglycine ester derivatives via C–N coupling under mild conditions. *Org. Chem. Front.* **2018**, *5*, 788–792.
7. Xiao, L.-J.; Zhu, Z.-Q.; Guo, D.; Xie, Z.-B.; Lu, Y.; Le, Z.-G. Coupling reaction of N-arylglycine esters with imides or amides for synthesis of α-substituted α-amino acid esters. *Synlett* **2018**, *29*, 1659–1663.
8. Li, J.J. Imide-containing synthetic drugs. In *Imides, Medicinal, Agricultural, Synthetic Applications and Natural Products Chemistry, Developments in Organic Chemistry*; Luzzio, F.A., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 353–366.

7.15. The Vicarious Nucleophilic Substitution Reaction of 2-Nitro-5,10,15,20-Tetraphenylporphyrin with p-Chlorophenoxyacetonitrile

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Tetrapyrrolic macrocycles are a versatile family of aromatic compounds with a unique set of physicochemical properties with potential application in a wide array of fields, such as solar cells, (chemo)sensors, supramolecular chemistry, and medicine [1]. *Meso*-tetraarylporphyrins bearing primary groups, namely nitro units, are excellent scaffolds to be used in further modifications of the porphyrin macrocycle, since they can participate in several synthetic approaches such as cycloadditions, nucleophilic addition, and nucle-

ophilic or electrophilic substitutions [2,3]. The vicarious nucleophilic substitution (VNS) of hydrogen is a valuable tool to functionalize nitro aromatic heterocyclic derivatives from their reaction with carbanions bearing leaving groups. This methodology is an efficient alternative to the nucleophilic aromatic substitution of halogens through SNAr addition–elimination [4]. Although the VNS reaction of metallo complexes of 2-nitroporphyrins with carbanions or active methylene compounds has already been explored by several authors and described in the literature, to the best of our knowledge, no examples were reported using free base 2-nitroporphyrins [5].

In this communication, we will discuss the VNS reaction of 2-nitro-5,10,15,20-tetraphenylporphyrin with *p*-chlorophenoxyacetonitrile and the gas-phase analysis of the compounds isolated.

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References

1. *Handbook of Porphyrin Science: Volumes 1–12*; Kadish, K.M., Smith, K.M., Guilard, R., Eds.; World Scientific Publishing Company Co.: Singapore, 2010.
2. Jaquinod, L. Functionalization of 5,10,15,20-tetra-substituted porphyrins. In *The Porphyrin Handbook*; Kadish, K.M., Smith, K.M., Guilard, R., Eds.; Academic Press: San Diego, CA, USA, 2000; Volume 1, pp. 212–222.
3. Cerqueira, A.; Moura, N.M.M.; Serra, V.V.; Faustino, M.A.F.; Tomé, A.C.; Cavaleiro, J.A.S.; Neves, M.G.P.M.S. β-Formyl- and β-Vinylporphyrins: Magic Building Blocks for Novel Porphyrin Derivatives *Molecules* **2017**, *22*, 1269.
4. Eddahmi, M.; Moura, N.M.M.; Bouissane, L.; Gamouh, A.; Faustino, M.A.F.; Cavaleiro, J.A.S.; Paz, F.A.A.; Mendes, R.F.; Lodeiro, C.; Santos, S.M.; Neves, M.G.P.M.S.; Rakib, E.M. New nitroindazolylacetonitriles: efficient synthetic access via vicarious nucleophilic substitution and tautomeric switching mediated by anions. *New J. Chem.* **2019**, *43*, 14355–14367.
5. Eddahmi, M.; Moura, N.M.M.; Ramos, C.I.V.; Bouissane, L.; Faustino, M.A.F.; Cavaleiro, J.A.S.; Rakib, E.M.; Neves, M.G.P.M.S. An insight into the vicarious nucleophilic substitution reaction of 2-nitro-5,10,15,20- tetraphenylporphyrin with p-chlorophenoxyacetonitrile: Synthesis and gas-phase fragmentation studies. *Arabian J. Chem.* **2020**, *13*, 5849–5863.

7.16. Novel Steroidal Arylidene Derivatives as Potential 5α-Reductase Inhibitors: Evaluation of Enzymatic Activity in Mouse Liver Microsomes by HPLC-DAS

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The enzyme 5α-reductase is responsible for converting testosterone into the more potent androgen 5α-dihydrotestosterone. The overproduction of 5α-dihydrotestosterone has an important role in the development of several male diseases such as benign prostatic hyperplasia and prostate cancer [1]. Steroidal derivatives have been studied and

applied as 5α-reductase inhibitors, such as finasteride, which currently clinically used for the symptomatic treatment of benign prostatic hyperplasia [2]. However, these molecules have shown relative low potency and several side effects [3]. In the present study, several steroidal arylidene derivatives previously synthesized by our group, including 4-azasteroids, as well as steroidal 5α,6α-epoxides, 3β,5α,6β-triols and 4-en-3,6-diones, were screened for 5α-reductase inhibitory activity using mice liver as an enzyme source (Figure 1) [4,5]. For this, testosterone was measured by an HPLC-DAD method partially developed and validated. IC_{50} values of the most active compounds were determined. Interestingly, the results showed that among the tested steroidal derivatives, four have an IC_{50} lower than the positive control, finasteride (IC_{50} = 91.49 nM). 16*E*-(2,4-dichlorobenzylidene)androst-4-ene-3,6,17-trione was the most potent compound (IC_{50} = 6.12 nM).

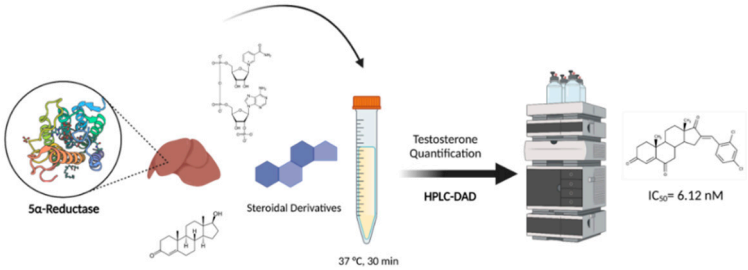

Figure 1. Methodology for the 5α-reductase inhibitory capacity evaluation of a series of novel steroidal arylidene derivatives using HPLC-DA. Created with BioRender.com.

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References

1. Azzouni, A.; Godoy, Y.; Li, Y.; Mohler, J. The 5α-reductase isozyme family: a review of basic biology and their role in human diseases. *Adv. Urol.* **2021**, *2012*, 530121.
2. Salvador, J.A.R.; Pinto, R.M.A.; Silvestre, S.M.J. Steroidal 5α-reductase and 17α-hydroxylase/17,20-lyase (CYP17) inhibitors useful in the treatment of prostatic diseases. Steroid Biochem. *Mol. Biol.* **2013**, *137*, 199–222.
3. Hirshburg, J.M.; Kelsey, P.A.; Therrien, C.A.; Gavino, A.C.; Reichenberg, J.S. Adverse effects and safety of 5α-reductase inhibitors (finasteride, dutasteride): a systematic review. *J. Clin. Aesthet. Dermatol.* **2016**, *9*, 56–62.
4. Brito, V.; Santos, A.O.; Almeida, P.; Silvestre, S. M. Novel 4-azaandrostenes as prostate cancer cell growth inhibitors: synthesis, antiproliferative effects and molecular docking studies. *Comptes Rendus Chim.* **2018**, *22*, 73–83.
5. Kumar, N.; Rungseevijitprapa, W.; Narkkhong, N.; Suttajit, M. 5α-Reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment. *J. Ethnopharmacol.* **2012**, *139*, 765–771.

7.17. Design and Synthesis of New Pyrazolo[3,4-d]pyrimidine Dimeric Structures from Substituted Pyrazoles

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Pyrazoles are an important family of aromatic dinitrogen heterocycles that are present in a number of bioactive compounds. The capacity to incorporate the pyrazole nucleus in different structures leads to diverse applications in different areas in particular in Medicinal

Chemistry. Substituted pyrazoles have been used as a starting material for the synthesis of pyrazolo[3,4-d]pyrimidines. They are important heterocycles due to their structural similarity with the purine scaffold [1,2].

In this work, a selection of pyrazoles 1, prepared from ethoxymethylenemalononitrile and substituted hydrazines, reacted with triethylorthoformate (TEOF), in the presence of acid catalysis, leading a new fused pyrazolo[3,4-d]pyrimidine (Figure 1). These compounds 2, isolated as salts, were generated through a cascade condensation–cyclization reaction, following a pathway similar to that previously reported for anthranilonitrile [3]. The synthetic approach will be discussed in detail. All the compounds were characterized by elemental analysis and spectroscopic (IR, 1H, 13C, 15N, HMQC and HMBC) techniques.

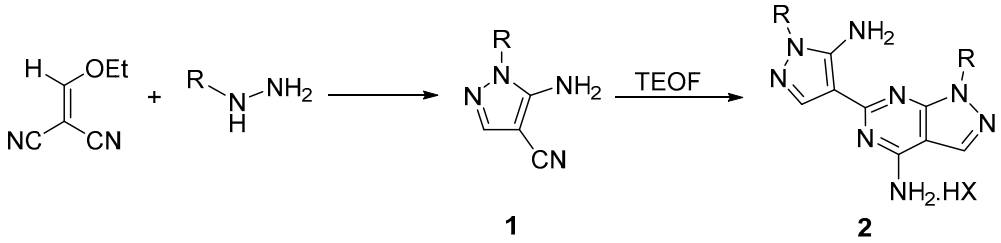

Figure 1. Synthesis of pyrazolo[3,4-*d*]pyrimidine **2** from pyrazole **1** with TEOF.

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References

1. Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y.N.; Al-Aizari, F.A.; Ansar, M. Synthesis and Pharmacological Activities of Pyrazole Derivatives: A Review. *Molecules* **2018**, *23*, 134, https://doi.org/10.3390/molecules23010134.
2. Schenone, S.; Radi, M.; Musumeci, F.; Brullo, C.; Botta, M. Biologically driven synthesis of pyrazolo[3,4-d]pyrimidines as protein kinase inhibitors: an old scaffold as a new tool for medicinal chemistry and chemical biology studies. *Chem. Rev.* **2014**, *14*, 7189–7238, https://doi.org/10.1021/cr400270z.
3. Marinho, E.; Araújo, R.; Proença, F. The reaction of anthranilonitrile and triethylorthoformate revisited: formation of dimeric and trimeric species. *Tetrahedron* **2010**, *66*, 8681–8689, https://doi.org/10.1016/j.tet.2010.09.013.

7.18. Novel β-Carboline Derivatives as Potential Anticancer Agents

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β-Carbolines are alkaloid-based compounds with a 9*H*-pyrido[3,4-*b*]indole scaffold, that show a wide range of biological activities, including anticancer activity [1]. On the other hand, tetrazole and triazole groups are relevant structures in Medicinal Chemistry

and have been associated to a wide range of biological activities [2,3]. Recently, a synthetic strategy to β-carbolines containing a tetrazole group was developed in our group, exploring the reactivity of nitrosoalkenes [4]. This strategy involves the hetero-Diels–Alder (HDA) reaction of nitrosoalkenes **2**, which was generated in situ from the corresponding α-halo-oximes (**1**), with indole giving an open-chain oxime **3** by an oxazine ring-opening and indole rearomatization process. The subsequent oxime reduction affords tryptophan analogues **4** which underwent Pictet–Spengler (PS) condensation with aldehydes and further oxidation leading to β-carbolines [4,5]. In this context, we decided to apply this synthetic strategy to obtain a new range of β-carbolines containing tetrazole (**5**) and triazole (**6**) moieties (Figure 1). The anticancer activity of the synthesized derivatives was evaluated against the human colon cancer cell lines (HCT116 wt, HCT116 −/− p53, SW837 and HT29), demonstrating that the presence of the triazole moiety in the C-3 position of β-carbolines severely hinders their anticancer activity ($IC_{50} \geq 32$ μM). Interestingly, the tetrazole β-carboline derivatives showed promising anticancer activity against HCT116 cell lines, with IC_{50} values ranging from 3.3 to 4.6 μM, as well as against HT29 cancer cell line with IC_{50} values ranging from 5.9 to 9.6 μM. Further details regarding the structure–activity relationships were discussed.

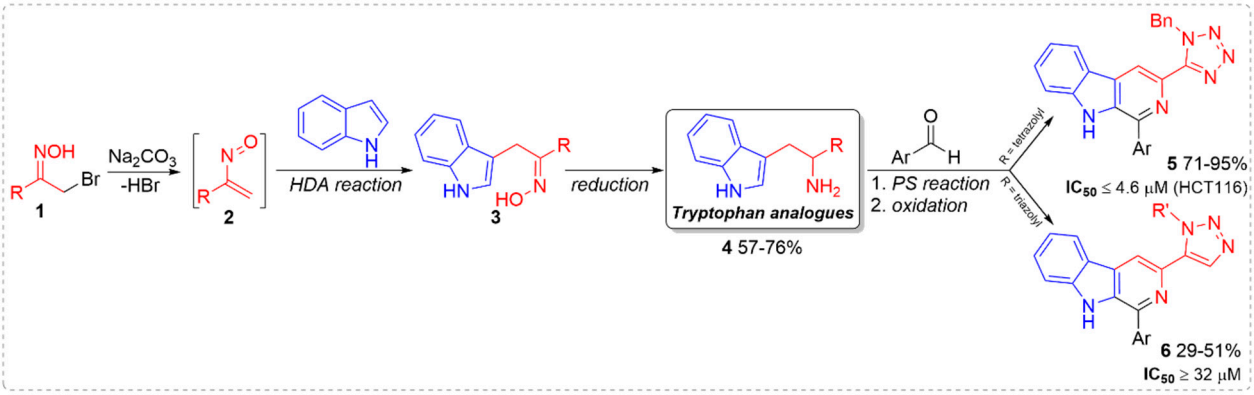

Figure 1. Synthetic route of β-carboline derivatives tested in human colon cancer cell lines.

Funding: The Coimbra Chemistry Centre (CQC) supported by the Portuguese Agency for Scientific Research, "Fundação para a Ciência e a Tecnologia" (FCT) through project UIDB/00313/2020 and UIDP/QUI/00313/2020, co-funded by COMPETE2020-UE. LAQV/REQUIMTE supported by the FCT through the project UIDB/50006/2020, co-founded by COMPETE2020-UE. João Ribeiro thanks the FCT for the PhD fellowship PD/BD/143160/2019 (MedChemTrain programme). We also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Luo, B.; Song, X. A comprehensive overview of β-carbolines and its derivatives as anticancer agents. *Eur. J. Med. Chem.* **2021**, *224*, 113688.
2. Sathish Kumar, S.; Kavitha, H.P. Synthesis and Biological Applications of Triazole Derivatives—A Review. *Mini Rev. Org. Chem.* **2013**, *10*, 40–65.
3. Kaushik, N.; Kumar, N.; Kumar, A.; Singh, K. U., Tetrazoles: Synthesis and Biological Activity. Immunol. Endocr. Metab. *Agents Med. Chem.* **2018**, *18*, 3–21.
4. Panice, M.R.; Lopes, S.M.M.; Figueiredo, M.C.; Goes Ruiz, A.L.T.; Foglio, M.A.; Nazari Formagio, A.S.; Sarragiotto, M.H.; Pinho e Melo, T.M.V.D. New 3-tetrazolyl-β-carbolines and β-carboline-3-carboxylates with anti-cancer activity. *Eur. J. Med. Chem.* **2019**, *179*, 123–132.
5. Lopes, S.M.M.; Palacios, F.; Lemos, A.; Pinho e Melo, T.M.V.D. Diels–Alder reactions of 3-(1H-tetrazol-5-yl)-nitrosoalkenes: synthesis of functionalized 5-(substituted)-1H-tetrazoles. *Tetrahedron* **2011**, *67*, 8902–8909.

7.19. Chiral 6,7-Bis(hydroxymethyl)-1H,3H-Pyrrolo[1,2-c]thiazoles as Novel p53-Activating Agents to Improve Colorectal Cancer Targeted Therapy

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Colorectal cancer (CRC) is the third most common cancer type and the second cause of cancer-related deaths worldwide [1]. Advances in understanding the pathogenesis of CRC demonstrated that impairment of the p53 pathway is a critical event in local and advanced CRCs. Thus, re-establishing p53 activity has become one of the most appealing anti-cancer therapeutic strategies. Recently, we disclosed a new p53-activating anticancer drug, (3*S*)-6,7-bis(hydroxymethyl)-5-methyl-3-phenyl-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (MANIO) [2]. MANIO demonstrated a notable selectivity to the p53 pathway, activating wild-type (WT) p53 and restoring WT-like function to mutant (mut) p53 in human cancer cells. The high efficacy of MANIO was further demonstrated in patient-derived cells and xenograft mouse models of CRC with no signs of undesirable side effects. Thus, MANIO represents a privileged anticancer drug compared to other p53-activating agents currently available. Herein, studies on lead optimization with the aim of obtaining MANIO-like derivatives with improved properties, particularly drug-likeness and pharmacokinetics, are presented (Figure 1).

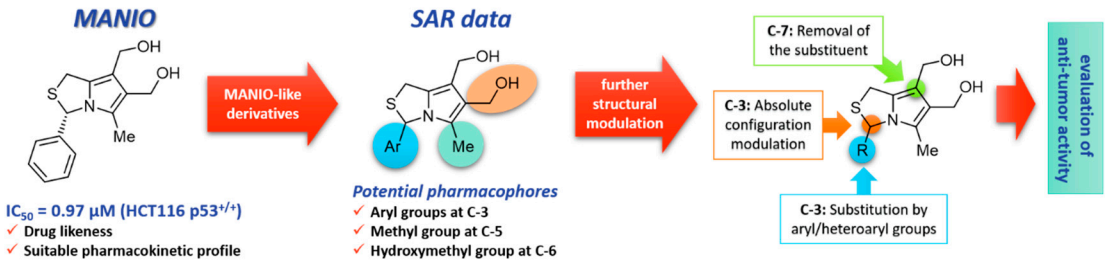

Figure 1. SAR-based structural modulation of MANIO.

Funding: This work was supported by PT National Funds (FCT/MCTES, Fundação para a Ciência e Tecnologia, and Ministério da Ciência, Tecnologia e Ensino Superior) via the projects UIDB/00313/2020 (CQC), UIDP/00313/2020 (CQC) and UIDB/50006/2020 (LAQV/REQUIMTE), co-funded by COMPETE2020-UE. J.L.P.R. thanks the FCT for the PhD fellowship PD/BD/143160/2019 (MedChemTrain programme)". The authors also acknowledge the UC-NMR facility for obtaining the NMR data (www.nmrccc.uc.pt, accessed on 19 August 2022).

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249.
2. Ramos, H.; Soares, M.I.L.; Silva, J.; Raimundo, L.; Calheiros, J.; Gomes, C.; Reis, F.; Monteiro, F.A.; Nunes, C.; Reis, S.; et al. A selective p53 activator and anticancer agent to improve colorectal cancer therapy. *Cell Rep.* **2021**, *35*, 108982.

7.20. Synthesis of Carbamate and Urea Derivatives under a Continuous Flow Process with in-Line Flash Chromatographic Purification

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Continuous flow procedures have become a widely used technique in organic and Medicinal Chemistry, allowing the application of old and novel chemistry in a safer, reproducible, and scalable fashion [1,2]. Additionally, this technique permits the application of in-line extraction and purification procedures, improving automation on synthesis with several gains in a laboratory of organic chemistry. Following previous reports on the application of continuous flow Curtius rearrangement and its significance in modern drug discovery [3,4], in this work, we focus on the synthesis of diaryl carbamate and urea derivatives through a continuous DPPA-mediated Curtius rearrangement procedure (Figure 1). Twelve compounds were easily obtained through the conjugation of diphenylacetic acid with different alcohols and amines as nucleophiles (Nuc-H). A comparison with the corresponding batch procedure was performed, and several variables involved with the reaction efficiency are discussed. Additionally, an automatic purification procedure was implemented through the in-line integration of a commercial automated flash chromatography system with the flow reactor, allowing the continuous synthesis and isolation of the desired products (Figure 1).

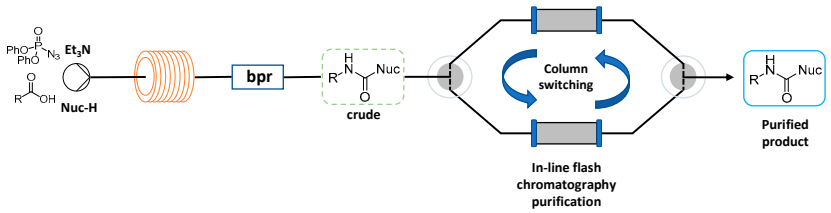

Figure 1. Continuous DPPA-mediated Curtius rearrangement procedure with in-line flash chromatography.

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References

1. Bonner, A.; Loftus, A.; Padgham, A. C.; Baumann, M. Forgotten and forbidden chemical reactions evitalized through continuous flow technology. *Org. Biomol. Chem.* **2021**, *19*, 7737–7753, https://doi.org/10.1039/D1OB01452H.
2. Donnelly, K.; Baumann, M. Scalability of photochemical reactions in continuous flow mode. *J. Flow Chem.* **2021**, *11*, 223–241, https://doi.org/10.1007/s41981--021-00168-z.
3. Baumann, M.; Baxendale, I.R.; Ley, S.V.; Nikbin, N.; Smith, C.D.; Tierney, J.P. A modular flow reactor for performing Curtius rearrangements as a continuous flow process. *Org. Biomol. Chem.* **2008**, *6*, 1577–1586, https://doi.org/10.1039/B801631N.

4. Ghosh, A.K.; Brindisi, M.; Sarkar, A. The Curtius Rearrangement: Applications in Modern Drug Discovery and Medicinal Chemistry. *ChemMedChem* **2018**, *13*, 2351–2373, https://doi.org/10.1002/cmdc.201800518.

7.21. Developing a Computer-Aided Drug Design (CADD) Approach to Discovery Lead-Like PMM2 Enzyme Activators for PMM2-CDG Therapy

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Amongst all the rare congenital disorders of glycosylation (CDG), PMM2-CDG is currently the most frequently occurring with >900 reported cases worldwide. It is caused by a genetically inherited deficiency in phosphomannomutase 2 (PMM2), which catalyzes the interconversion of Man-6-P into Man-1-P, the last being required for post-translational *N*-glycosylation. As glycosylation is essential for the correct function of several glycoconjugates, defects in these pathways lead to multisystem diseases that cause a variety of symptoms and phenotypes, ranging from very mild to extremely severe. As of today, no effective treatment is available for PMM2-CDG [1]. Previous functional characterization studies of disease-causing mutations described in PMM2-CDG patients brought forth the possibility of designing a targeted therapy using pharmacological chaperones (PC) to rescue loss-of-function modifications in the abnormal PMM2 enzyme [2]. The aim of this work is to develop a computer-aided drug design (CADD) approach to discover possible PCs for PMM2 activation. Two machine learning strategies were developed: (1) to build a quantitative structure–activity relationship (QSAR) classification model able to predict the interaction of drug-like molecules with PMM2 and, afterwards, (2) to build yet another QSAR regression model to estimate a theoretical value for IC50 (half maximal inhibitory concentration). In order to build these prediction models, we used experimentally validated compound datasets whose interaction with PMM2, namely protein stability (Tm) and IC50, had been previously calculated by high-throughput screening approaches. The best QSAR models will be used as computational tools to run data libraries with thousands of FDA-approved and drug-like compounds to search for and select molecules with the desired PC profile. The most promising results will be submitted to molecular modulation and structural studies to further characterize the properties of the protein–ligand interaction, and experimental validation will be conducted in order to confirm whether the models are accurate and to further analyze the activity of selected molecules on PMM2 (Figure 1).

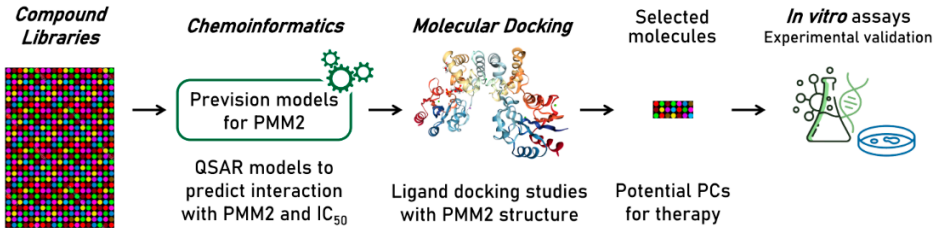

Figure 1. Simplified workflow for CADD approach to screen compounds for potential PCs for PMM2-CDG therapy.

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References

1. Brasil, S.; Pascoal, C.; Francisco, R.; Marques-da-Silva, D.; Andreotti, G.; Videira, P.A.; Morava, E.; Jaeken, J.; Ferreira, V.R. CDG Therapies: From Bench to Bedside. *Int. J. Mol. Sci.* **2018**, *27*, 1304. https://doi.org/10.3390/ijms19051304.
2. Yuste-Checa, P.; Brasil, S.; Gámez, A.; Underhaug, J.; Desviat, L.R.; Ugarte, M.; Pérez-Cerdá, C.; Martinez, A.; Pérez, B. Pharmacological Chaperoning: A Potential Treatment for PMM2-CDG. *Hum. Mut.* **2017**, *38*, 160–168. https://doi.org/10.1002/humu.23138.

7.22. Determination of Fatty Acids Profile Produced by Marine-Derived Actinobacteria from Estremadura Spur Pockmarks

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Ocean environments constitute an important source of biodiversity, harboring marine life forms capable of producing a variety of molecules with unique characteristics, unparalleled biochemical diversity and structural complexity. Compounds produced by bacteria cover a wide structural range, including metabolites of the fatty acid biosynthetic pathway. These are carboxylic acids with a long aliphatic chain, saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from C4 to C28 [1]. In either form, fatty acids are important structural components of cells with several biotechnological applications, including fuel, food sources for animals, cosmetics and pharmaceutical agents.

Actinobacteria isolated from sediments collected off the coast of Portugal in the Estremadura Spur pockmarks fields (200–400 m depth) revealed the presence of fatty acids, suggesting that these strains have evolved to produce these chemical compounds in higher abundance than strains from other locations [2,3]. In this work, a detailed investigation

of the fatty acids profile produced by 55 Estremadura Spur Actinobacteria strains was performed by GC/MS after transesterification of the lipidic extract toward the respective methyl esters.

Our results revealed that strains from *Micromonospora*, *Streptomyces*, *Saccharopolyspora*, *Actinomadura*, *Nocardiopsis*, *Saccharomonospora*, and *Stackebrandtia* genera produce SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), cyclo fatty acids, odd fatty acids and BCFA (branched chain fatty acids). The majority are BCFA (41%), MUFA (33%) and SFA (30%). For the lipid profile of BCFA regarding the branch position, iso series are the most abundant. The MUFA profile exhibited ω9 (26%), ω7 (5%), and ω6 and ω5 families in lower amounts.

This study demonstrates that the Estremadura Spur Actinobacteria are a rich source of fatty acids with potential applications in biotechnology.

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References

1. Alhassan, M.; Bello, A.M.; Suleiman, M.; Safiya, A.M.; Grba, A.A.; Nasiru Y. Comparative Fatty Acids Composition of Cashew, Fenugreek and Moringa Seed Oils. *J. Chem. Sci.* **2019**, *2*, 321–332, https://doi.org/10.34198/ejcs.2219.321332.
2. Pinto-Almeida, A.; Bauermeister, A.; Lupino, L.; Grilo, I.R.; Oliveira, J.; Sousa, J.R.; Petras, D.; Rodrigues, C.F.; Prieto-Davó, A.; Tasdemir, D.; et al. The Diversity, Metabolomics Profiling, and the Pharmacological Potential of Actinomycetes Isolated from the Estremadura Spur Pockmarks (Portugal). *Mar. Drugs*, **2021**, *20*, 21, https://doi.org/10.3390/md20010021.
3. Prieto-Davó, A.; Dias, T.; Gomes, S.E; Rodrigues, S.; Parera-Valadez, Y.; Borralho, P.M.; Pereira, F.; P. Rodrigues, C.M.; Santos-Sanches, I.; Gaudêncio, S.P. The Madeira Archipelago as a Significant Source of Marine-derived Actinomycete Diversity with Anticancer and Antimicrobial Potential. *Front. Microbiol.* **2016**, *7*, 1594, https://doi.org/10.3389/fmicb.2016.01594.

7.23. Valorization of Macaronesia Beach-Cast Seaweeds: Secondary Metabolites and Antiaging Activity

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Beach-cast seaweeds are a seasonal phenomenon consisting of the accumulation of large tons of algae on beaches, which is unpleasant for beach users and affects the tourism industry, mainly because tourists often interpret stranded natural litter as lowering beach

quality, especially if the material starts to decompose [1]. These beach casts are always variable mixtures of different species of seagrass and seaweeds [2]. The present work aimed to contribute to the valorization of this biomass by studying its chemical composition and bioactivities that reveal its potential in the pharmaceutical and/or cosmeceutical industries.

The beach-cast seaweed studied here was collected at Playa de Las Canteras, Las Palmas, Gran Canaria, it and has the following composition: *Stypocaulon scoparium* (68.7%) and *Lobophora variegata* (14.4%), both brown algae, the green alga *Cymopolia barbata* (13.4%) and red algae from *Liagora genus* (3.5%). The methanol extract, obtained from dry material, was fractionated by solubility in different solvents. The extract and fractions were evaluated for their antiaging activity.

The most active fractions were fractionated by different chromatographic techniques, obtaining four pure compounds. The use of spectroscopic techniques (1D and 2D NMR, MS) allowed the elucidation of the chemical structure (Figure 1) of three already known compounds (compounds **1**–**3**) [3] and one described for the first time in the literature (compound **4**). Lobophorol B exhibited antioxidant activity being a weak inhibitor of tyrosinase and cholinesterase. All the experimental results and their discussion will be presented.

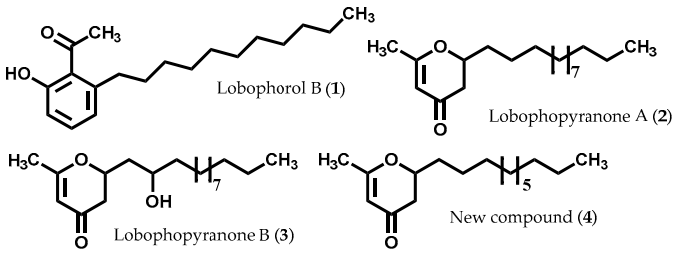

Figure 1. Chemical structure of compounds isolated from beach-cast seaweed.

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References

1. van Beukering, P.J.H.; Cesar, H.S.J. Ecological Economic Modeling of Coral Reefs: Evaluating Tourist Overuse at Hanauma Bay and Algae Blooms at the Kihei Coast, *Hawai'i. Pac. Sci.* **2004**, *58*, 243–260.
2. Zárate, R.; Portillo, E.; Teixidó, S.; Carvalho, M.A.P.; Nunes, N.; Ferraz, S.; Seca, A.M.L.; Rosa, G.P.; Barreto, M.C. Pharmacological and Cosmeceutical Potential of Seaweed Beach-Casts of Macaronesia *Appl. Sci.* **2020**, *10*, 5831.
3. Vieira, C.; Gaubert, J.; de Clerck, O.; Payri, C.; Culioli, G.; Thomas, O.P. Biological activities associated to the chemodiversity of the brown algae belonging to genus Lobophora (Dictyotales, Phaeophyceae). *Phytochem. Rev.* **2017**, *16*, 1–17.

7.24. Identification of Phthalates in the Angolan Diospyros Batocana Medicinal Plant—Natural Products or Contaminants?

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Phthalates, phthalic acid esters, are a group of lipophilic chemical compounds widely produced in the industry [1]. They have demonstrated many toxic effects and can cause endocrine disorders in humans. Despite this, many investigations found phthalates in natural sources, such as plants, bacteria and fungi, suggesting their possible natural biosynthesis [1]. These studies have raised doubts about their origin and classification as natural pollutants or metabolites. Indeed, according to these studies, phthalates should not be treated solely as manufactured pollutants simply because they have been widely synthesized and used. Instead, they should be presented as natural metabolites with recognized biological activities [1].

Diospyros batocana, the plant studied in this work, was collected in Angola, in the commune of Leua, a small village of Soba-Kapalu, Moxico. This specie belongs to the genus *Diospyros*, the family Ebenaceae, which includes 500 to 600 species [2]. Plants, including *Diospyros batocana*, are traditionally formulated in Africa and prescribed extracts and decoctions to treat several diseases. Several have been extensively tested for their multiple pharmacological activities [2].

In this paper, we describe the identification of phthalic compounds in non-polar and polar fractions (samples DBCHI-1 to 31 (fractions 1–58)] and samples [DBCHII-1 to 24 (fractions 1–128)), which were isolated by vacuum liquid chromatography and silica gel chromatography from hexane (HEX) and acetone extracts (ACE) of *Diospyros batocana* leaves, respectively. NMR and GC-MS were used to carry out chemical analyses. The chemical constituents were identified based on the spectral data obtained and compared with literature data. The di-2-Ethylhexyl phthalate (DEP) (Figure 1a) obtained from the HEX fractions [DBCHI-1 (F1 to 5), DBCHI-3 (F9 to 14) and DBCHI-4 (F15)] varied near 20.80% to 80.67%. In contrast, it was present in levels ranging from 31.05% to 55.02% in the ACE fractions (DBCHII-6 (F16-17-18) and DBCHII-11 (F18)). Another phthalate derivative, dibutyl phthalate (DBP) (Figure 1b), was also detected in a proportion of 30.42% in DBCHII-34 (F61 to F65).

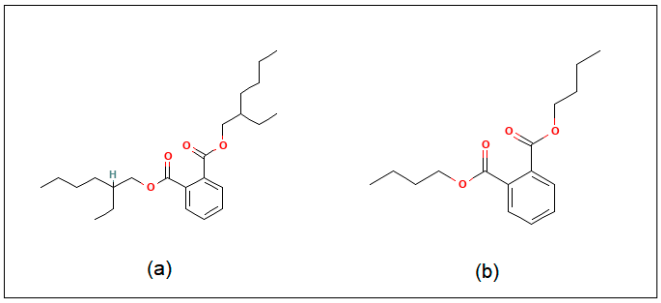

Figure 1. Structures of (**a**) Di-2-ethylhexyl phthalate identified in the HEX fractions (DBCHI-1 (F1 to 5), DBCHI-3 (F9 to 14) and DBCHI-4 (F15)) and ACE fractions ((DBCHII-6 (F16-17-18) and DBCHII-11 (F18)); (**b**) Dibutyl phthalate identified in ACE fractions DBCHII-34 (F61 to F65).

These preliminary results suggest two possible approaches or hypotheses for the origin of phthalates in *Diospyros* leaf extracts. The chemicals are absorbed from the atmosphere, soils and contaminated waters in Angola, where the plant leaves were harvested. Phthalates can quickly be released into the various Angolan ecosystems, leading to a probable uptake and accumulation by medicinal and food plants. This accumulation is mainly related to waste disposal problems in developing countries [7]. Because of their human health and environmental toxicities, the medicinal plants need to be monitored for their phthalate contents. Therefore, competent authorities should implement mandatory quality and safety

measures and regulations for herbal products, their sources, manufacturing and plastic packaging. The alternative hypothesis is that phthalates are indeed biosynthesized by *Diopyros batocana*. The published literature indicates that they are also natural compounds and serve as biologically active substances for competitive selection with a claimed allelopathic activity that could facilitate the dominance of plants or algae capable of producing them [1,3–6]. These phthalate derivatives are probably synthesized through the shikimic acid pathway and are a barrier against biotic and abiotic factors [8]. In conclusion, synthetic and natural phthalates are widely distributed around us, and they deserve special attention regarding their origin, possible use or reduction in toxic production and environmental contamination.

References

1. Huang, L.; Zhu, X.; Zhou, S.; Cheng, Z.; Shi, K.; Zhang, C.; Shao, H. Phthalic acid esters: natural sources and biological activities. *Toxins* **2021**, *13*, 495. https://doi.org/10.3390/TOXINS13070495.
2. Net, S.; Sempere, R.; Delmont, A.; Paluselli, A.; Ouddane, B. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. *Environ. Sci. Technol.* **2015**, *49*, 4019–4035. https://doi.org/10.1021/es505233b.
3. Thiemann, T. Isolation of phthalates and terephthalates from plant material—Natural products or contaminants? *Open Chem. J.* **2021**, *8*, 1–36. https://doi.org/10.2174/1874842202108010001.
4. Roy, R. Bioactive natural derivatives of phthalate ester. *Crit. Rev. Biotechnol.* **2020**, *40*, 913–929. https://doi.org/10.1080/07388551.2020.1789838.
5. Zhang, H.; Hua, Y.; Chen, J.; Li, X.; Bai, X.; Wang, H. Organism-derived phthalate derivatives as bioactive natural products. *J. Environ. Sci. Health. C. Environ. Carcinog. Ecotoxicol. Rev.* **2018**, *36*, 125–144. https://doi.org/10.1080/10590501.2018.1490512.
6. Ortiz, A.; Sansinenea, E. Di-2-ethylhexylphthalate may be a natural product, rather than a pollutant. *J. Chem.* **2018**, *7*, 6040814. https://doi.org/10.1155/2018/6040814.
7. Ferronato, N.; Torretta, V. Waste mismanagement in developing countries: A Review of Global Issues. *Int. J. Environ. Res. Public Health* **2019**, *16*, 1060. https://doi.org/10.3390/IJERPH16061060.
8. Chen, C.Y. Biosynthesis of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) from red alga—Bangia atropurpurea. *Water Res.* **2004**, *38*, 1014–1018. https://doi.org/10.1016/J.WATRES.2003.11.029.

7.25. Kaempferol Derivatives from Hedychium gardnerianum—Unveiling the Potential of an Invasive Plant

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The therapeutic properties of plants and of their secondary metabolites are a current research topic of great interest. Considering that Hedychium species are used in folk medicine around the globe [1], Hedychium gardnerianum Sheppard ex Ker Gawl., an extremely aggressive invasive plant in Hawaii [2] and in Azores [3], was selected and phytochemically studied in order to search for natural compounds with interesting biological activities. Maceration of the dried aerial parts of the plant (200 g) took place with ethanol 96% (2 L) as solvent, providing an ethanolic extract of 14.30 g. Through liquid–liquid partition, fractionation of the extract originated the hexane, ethyl acetate and aqueous fractions. The hexane fraction was subjected to column chromatography and

thin layer chromatography (TLC), leading to the isolation of three pure compounds that were analyzed by nuclear magnetic resonance (NMR) and mass spectrometry (MS). The ethanolic extract and its fractions were tested regarding their antioxidant properties (ABTS and DPPH assays), with only the ethanolic extract (IC_{50} = 34.18 ± 0.97 μg/mL in ABTS assay and IC50 = 6.21 ± 1.04 μg/mL in DPPH assays) and the ethyl acetate fraction (IC_{50} = 20.38 ± 0.47 μg/mL in ABTS) reporting interesting results. The NMR and MS data enabled the identification of three flavonols (Figure 1), two of them new in the Hedychium genus, i.e., kaempferol 7,4′-dimethyl ether (1) and kaempferol 3,7,4′-trimethyl ether (2), and one new in the Zingiberaceae family, i.e., kaempferol 3,5,7,4′-tetramethyl ether (3). Flavonols are known for their bioactive activities; e.g., kaempferol 7,4′-dimethyl ether (1) has reported antioxidant activity of 187.28 ± 1.82 μg/mL IC_{50} value in DPPH assay [4], and kaempferol 3,5,7,4′-tetramethyl ether (3) demonstrated antidiabetic properties [5]; thus, the continuing study of Hedychium gardnerianum fractions is promising in the near future.

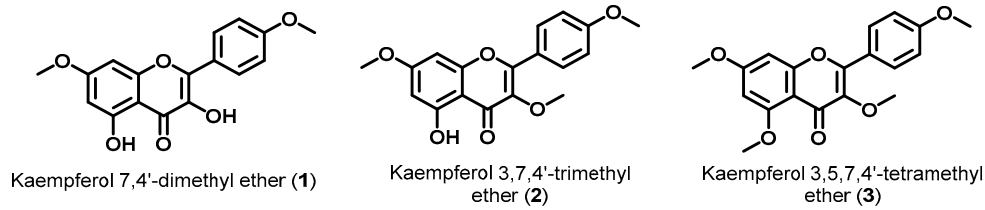

Figure 1. The three flavonols isolated from *Hedychium gardnerianum*.

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References

1. Tavares, W.R.; Barreto, M.C.; Seca, A.M.L. Uncharted source of medicinal products: The case of the Hedychium genus. *Medicines* **2020**, *7*, 23. https://doi.org/10.3390/medicines7050023.
2. Minden, V.; Hennenberg, K.J.; Porembski, S.; Boehmer, H.J. Invasion and management of alien Hedychium Gardnerianum (kahili ginger, Zingiberaceae) alter plant species composition of a montane rainforest on the island of Hawai'i. *Plant Ecol.* **2010**, *206*, 321–333. https://doi.org/10.1007/s112558-009-9645-9.
3. Pereira, M.J.; Eleutério, T.; Meirelles, M.G.; Vasconcelos, H.C. Hedychium gardnerianum Sheph. ex Ker Gawl. from its discovery to its invasive status: A review. *Bot. Stud.* **2021**, *62*, 11. https://doi.org/10.1186/s40529-021-00318-5.
4. Bian, Q.-Y.; Wang, S.-Y.; Xu, L.-J.; Chan, C.-O.; Mok, D.-K.; Chen, S.-B. Two new antioxidant diarylheptanoids from the fruits of Alpinia oxyphylla. *J. Asian Nat. Prod. Res.* **2013**, *15*, 1094–1099. https://doi.org/10.1080/10286020.2013.816297.
5. Matsuda, H.; Kogami, Y.; Nakamura, S.; Sugiyama, T.; Ueno, T.; Yoshikawa, M. Structural requirements of flavonoids for the adipogenesis of 3T3-L1 cells. *Bioorg. Med. Chem.* **2011**, *19*, 2835–2841. https://doi.org/10.1016/j.bmc.2011.03.040.

7.26. Synthesis and Characterization of Polymersomes with a Glycosylated Xanthone for Glioma

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Polymersomes (PMs) are artificial vesicles enclosing an aqueous cavity, resulting from the self-assembly of amphiphilic copolymers [1]. These sequences of polymers depend on the number of polymer chains, and they originate versatile structures that can encapsulate hydrophilic or hydrophobic drugs [2]. PMs vary in charge and dimension, are biocompatible and biodegradable, have demonstrated low in vivo toxicity, and showed better physical and chemical properties than liposomes [3].

The purpose of this study was to develop a delivery system based on PMs capable of delivering a synthetic xanthone glycoside (XGA), with proven antiproliferative activity against U251M, U373, and U87-MG cell lines with GI50 values between 0.19 and 0.55 μM [4].

The XGA was synthesized by the Michael reaction method: 3,6-dihydroxyxanthone reacted with 2,3,4,6- tetra-O-acetyl-α-D-glucopyranosyl bromide in the presence of potassium carbonate and tetrabuthylammonium bromide in a byphasic solvent system (water:chloroform) [4].

Two different types of PEGs, PEG5000 and PEG2000, were used for the synthesis of the amphiphilic copolymers PEG-PCL. The PEG-PCL were synthesized by the ring-opening polymerization method. The PCL (hydrophobic block) and stannous octoate (catalyst) were added to the dry PEG (hydrophilic block) and heated in a microwave oven. In order to purify the synthesized copolymer, the crude product was dissolved in an adequate amount of chloroform, and the synthesized copolymer was precipitated by adding cold diethyl ether. The characterization of the copolymers was performed by nuclear magnetic resonance (NMR).

PMs with and without XGA were prepared by the film rehydration method. The entrapment efficiency (EE) was determined by HPLC (265 nm). Both formulations have high drug EE (ca 99%).

These formulations were also characterized by dynamic light scattering (DLS). The size stability results showed that PMs without XGA had a mean size around 100 nm; in comparison, the PMs with XGA showed a mean size of 200 nm. All formulations showed negative zeta potential values and good physical stability after preparation as well as after 1, 7, and 14 days. The transmission electronic microscopy (TEM) technique was used to obtain images of the particles after PMs hydration to evaluate their morphology. The cell growth of the U251M, U373 and U87-MG cell lines after exposure to the prepared PMs will be evaluated as well as the blood–brain barrier permeability.

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References

1. Zhang, X.; Chen, L.; Chai, W.; Lian, X.-Y.; Zhang, Z.; A unique indolizinium alkaloid streptopertusacin A and bioactive bafilomycins from marine-derived Streptomyces sp. HZP-2216E. *Phytochemistry* **2017**, *144*, 119–126.

2. Mohammadi, M.; Ramezani, M.; Abnous, K.; Alibolandi, M.; Biocompatible polymersomes-based cancer theranostics: Towards multifunctional nanomedicine. *IJP* **2017**, *519*, 287–303.
3. Chandrawati, R.; Caruso, F.; Biomimetic liposome and polymersome-based multicompartmentalized assemblies. *Langmuir* **2012**, *28*, 13798–807.
4. Alves, A.; Correia-da-Silva, M.; Nunes, C.; Campos, J.; Sousa, E.; Silva, P.M.A.; Bousbaa, H.; Rodrigues, F.; Ferreira, D.; Costa, P.C; Pinto, M.; Discovery of a New Xanthone against Glioma: Synthesis and Development of (Pro)liposome Formulations. *Molecules* **2019**, *24*, 409, https://doi.org/10.3390/molecules24030409.

7.27. Evaluation of COX-2 Inhibitory Activity by Hydroxylated and Methoxylated 2-Styrylchromones

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Cyclooxygenase (COX), also known as prostaglandin H synthase, is the key isoenzyme in the synthesis of prostanoids, namely prostaglandins (PG) and thromboxanes from arachidonic acid. COX-2 is an inducible isoform of the enzyme and is expressed in cells involved in inflammation. Thus, the modulation of COX-2 enzyme activity is essential for the regulation of the inflammatory response and symptoms [1]. 2-Styrylchromones (2-SC) are derived from chromones and are characterized by a styryl group attached to C-2 of the chromone core and have demonstrated anti-inflammatory potential [2].

Therefore, this work intended to uncover the effect of a panel of seven structurally related hydroxylated and methoxylated 2-SC (Figure 1) on COX-2 enzyme activity inhibition. For this purpose, an in vitro non-cellular assay based on the fluorometric detection of PGG_2 was applied [3].

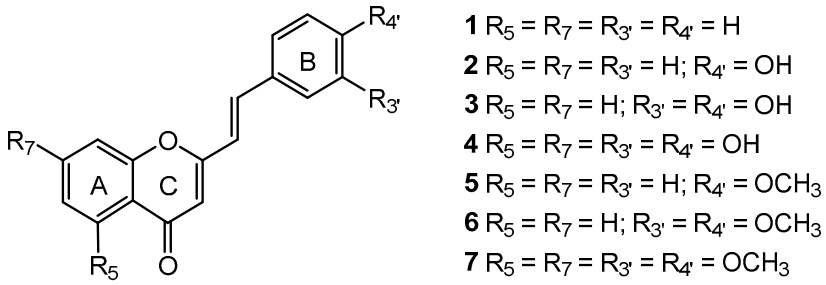

Figure 1. Chemical structure of the tested 2-SC.

The hydroxylated 2-SC **3** and **4** were the most active compounds (IC_{50} < 5 μM), whereas the methoxylated 2-SC were not effective. The obtained results suggest that the OH groups present on the B-ring are crucial for the COX-2 enzyme activity inhibition, especially the presence of the catechol group. In conclusion, the obtained results allowed the establishment of a structure–activity relationship and showed that the 2-SC scaffold is promising for the development of anti-inflammatory agents.

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References

1. Cipollone, F.; Cicolini, G.; Bucci, M. Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. *Pharmacol. Ther.* **2008**, *118*, 161–180, https://doi.org/10.1016/j.pharmthera.2008.01.002.
2. Lucas, M.; Freitas, M.; Silva, A.M.S.; Fernandes, E.; Ribeiro, D. Styrylchromones: Biological Activities and Structure-Activity Relationship. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 2804521, https://doi.org/10.1155/2021/2804521.
3. Sağlık, B.N.; Osmaniye, D.; Levent, S.; Çevik, U.A.; Çavuşoğlu, B.K.; Özkay, Y.; Kaplancıklı, Z.A. Design, synthesis and biological assessment of new selective COX-2 inhibitors including methyl sulfonyl moiety. *Eur. J. Med. Chem.* **2021**, *209*, 112918, https://doi.org/10.1016/j.ejmech.2020.112918.

7.28. Evaluation of Antiproliferative and Apoptotic Effects of Flavonoids in Osteosarcoma In Vitro Models

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Osteosarcoma (OS) is the most common childhood bone cancer. Compared to other tumors, OS shows high genetic heterogeneity, and selective pressure enhances phenotypic shifting across the tissue–time continuum [1]. Flavonoids are used in medicinal products to lessen disease manifestations as venous insufficiency, thrombosis/platelet dysfunction, osteoporosis or (corona)viral infection [2–5]. Synthetic polyphenols include dimefline (respiratory stimulant), flavodilol (anti-hypertensive), and flavoxate and terflavoxate (anti-spasmodic). It is noteworthy that polyphenol anticancer agents have already been developed, including cyclin-dependent kinase (CDK) inhibitors flavopiridol, voruciclib, riviciclib, and mitogen-activated protein (MAP) kinase inhibitor PD 98059, and the flavonoid isoquercetin [6]. In vitro, flavonoids as fisetin or 3′,4′-dihydroxyflavonol were also shown to inhibit osteosarcoma proliferation [7,8].

The aim of the present work was to evaluate the in vitro antiproliferative activity of a group of flavonoids on OS in vitro. For this, OS cell lines MG-63 and Saos-2 were incubated with flavonoids presenting various substituents (methoxy, chlorine, iodine, and alkyl) for 48 h, and, subsequently, cell viability was investigated upon incubation with WST-8 reagent, which was followed by spectrophotometric measurement at 450 nm. Moreover, potential apoptotic effects were investigated with Annexin-V/propidium iodide flow cytometric assay (24 h flavonoid incubation).

The obtained results suggest that substituents in unusual positions such as at the 6 or 8-position of the C ring contribute to the cytotoxic effect. The data also indicate an involvement of apoptosis in the cytotoxic action of 3,3′,4′,5,7,8-hexahydroxyflavone. Although antiproliferative effects can be achieved by multiple mechanisms, these data point to substituents/positions with more relevant effects and call for additional research on the multiple mechanisms of action.

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References

1. Brown, H.K.; Schiavone, K.; Gouin, F.; Heymann, M.F.; Heymann, D. Biology of Bone Sarcomas and New Therapeutic Developments. *Calcif. Tissue Int.* **2018**, *102*, 174–195, https://doi.org/10.1007/s00223-017-0372-2.
2. Belczak, S.Q.; Sincos, I.R.; Campos, W.; Beserra, J.; Nering, G.; Aun, R. Veno-active drugs for chronic venous disease: A randomized, double-blind, placebo-controlled parallel-design trial. *Phlebology* **2014**, *29*, 454–60, https://doi.org/10.1177/0268355513489550.
3. Zwicker, J.I.; Schlechter, B.L.; Stopa, J.D.; Liebman, H.A.; Aggarwal, A.; Puligandla, M.; Caughey, T.; Bauer, K.A.; Kuemmerle, N.; Wong, E.; Wun, T.; McLaughlin, M.; Hidalgo, M.; Neuberg, D.; Furie, B.; Flaumenhaft, R.; CATIQ, Investigators11. Targeting protein disulfide isomerase with the flavonoid isoquercetin to improve hypercoagulability in advanced cancer. *JCI Insight* **2019**, *4*, e125851, https://doi.org/10.1172/jci.insight.125851.
4. Wang, Z.; Wang, D.; Yang, D.; Zhen, W.; Zhang, J.; Peng, S. The effect of icariin on bone metabolism and its potential clinical application. *Osteoporos. Int.* **2018**, *29*, 535–544, https://doi.org/10.1007/s00198-017-4255-1.
5. Mouffouk, C.; Mouffouk, S.; Mouffouk, S.; Hambaba, L.; Haba, H. Flavonols as potential antiviral drugs targeting SARS-CoV-2 proteases (3CLpro and PLpro), spike protein, RNA-dependent RNA polymerase (RdRp) and angiotensin-converting enzyme II receptor (ACE2). *Eur. J. Pharmacol.* **2021**, *891*, 173759, https://doi.org/10.1016/j.ejphar.2020.173759.
6. Buonerba, C.; De, Placido, P.; Bruzzese, D.; Pagliuca, M.; Ungaro, P.; Bosso, D.; Ribera, D.; Iaccarino, S.; Scafuri, L.; Liotti, A.; Romeo, V.; Izzo, M.; Perri, F.; Casale, B.; Grimaldi, G.; Vitrone, F.; Brunetti, A.; Terracciano, D.; Marinelli, A.; De, Placido, S.; Di, Lorenzo, G. Isoquercetin as an Adjunct Therapy in Patients With Kidney Cancer Receiving First-Line Sunitinib (QUASAR): Results of a Phase I Trial. *Front. Pharmacol.* **2018**, *9*, 189, https://doi.org/10.3389/fphar.2018.00189.
7. Ferreira de Oliveira, J.M.P.; Pacheco, A.R.; Coutinho, L.; Oliveira, H.; Pinho, S.; Almeida, L.; Fernandes, E.; Santos, C. Combination of etoposide and fisetin results in anti-cancer efficiency against osteosarcoma cell models. *Arch. Toxicol.* **2018**, *92*, 1205–1214, https://doi.org/10.1007/s00204-017-2146-z.
8. Ferreira de Oliveira, J.M.P.; Almeida, J.F.D.; Martins, M.; Proença, C.; Oliveira, H.; Fernandes, E.; Santos, C. 3′,4′-Dihydroxyflavonol Modulates the Cell Cycle in Cancer Cells: Implication as a Potential Combination Drug in Osteosarcoma. *Pharmaceuticals* **2021**, *14*, 640, https://doi.org/10.3390/ph14070640.

7.29. Structural Characterization of a G-Quadruplex Aptamer and Their Ligands

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Nucleic acid aptamers, namely AS1411, have demonstrated some advantages over monoclonal antibodies, such as small size, high binding affinity, specificity, good biocompatibility, stability, and low immunogenicity. 1 They can be applied in different delivery systems with direct therapeutic potential, such as drug carriers to facilitate specific cellular recognition and uptake [1]. Therefore, new aptamers derivatives must arise to make G-rich aptamers widely used in the preclinical and clinical applications [2]. AT11-B1 is a variant sequence of AT11 (modified version of AS1411), in which we remove one thymine from the bulge [3] (Figure 1A). Herein, we have studied the G-quadruplex (G4) formation and stabilization using PhenDC3, PDS, BRACO-19, TMPyP4 and 360A ligands by fluorescence resonance energy transfer (FRET-melting) circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies (Figure 1B). The spectra suggest predominant parallel G4 topology after supplementation of KCl and ligands, and there were no changes of signals or bands upon the addition of an excess of ligands maintaining the structure. PhenDC3 stabilizes the structure at temperatures of more than 30 °C. All the ligands exhibit high affinity toward AT11-B1 G4 as well as the respective complexes against nucleolin (NCL), suggesting that the ligands do not negatively affect the recognition of the NCL by AT11-B1 G4. NMR studies showed that AT11-B1 forms a G4 containing four G-tetrad layers. AT11-B1 G4/PhenDC3 is only observed at a 1:4 DNA/ligand ratio, which corresponds to the possibility of four PhenDC3 molecules binding to a G4. The in silico studies show that all ligands bind AT11-B1 G4, namely, by stacking interactions, except PDS that bind to the loop/groove interface; also, molecular dynamics simulations revealed that NCL interacts with the AT11-B1 G4 structure through the RNA-binding domain (RBD) 2 and the 12-residue linker between RBD1,2. Moreover, AT11-B1 G4 is internalized into a nucleolin-positive tongue squamous cell carcinoma cell line.

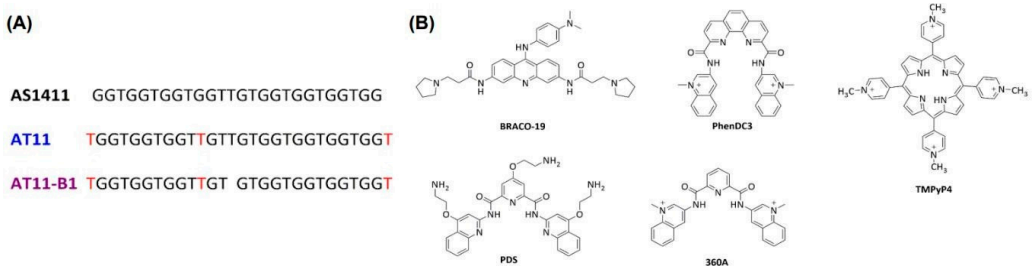

Figure 1. (**A**) AT11-B1, AT11 and AS1411 (modifications shown in red) sequences. (**B**) Chemical structure of the G4 ligands.

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References

1. Zhou, J.; Rossi, J. Aptamers as targeted therapeutics: current potential and challenges. *Nat. Rev. Drug Discov.* **2017**, *16*, 181–202, Https://doi.org/10.1038/NRD.2016.199.
2. Tucker, W.O.; Shum, K.T.; Tanner, J.A. G-Quadruplex DNA Aptamers and Their Ligands: Structure, Function and Application. *Curr. Pharm. Des.* **2012**, *18*, 2014–2026, Https://doi.org/10.2174/138161212799958477.
3. Do, N.Q.; Chung, W.J.; Hong, T.; Truong, A.; Heddi, B.; Phan, A.T. G-quadruplex structure of an anti- proliferative DNA sequence. *Nucleic Acids Res.* **2017**, *45*, 7487–7493, Https://doi.org/10.1093/NAR/GKX274.

7.30. Chiral Derivatives of Xanthones: Enantioselectivity in the Reversal Antimicrobial Resistance Mechanisms

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Efflux pumps are membrane transporters ubiquitous to bacteria that are responsible for the transport of xenobiotics to the outside of the bacterial cell. It is also an antimicrobial resistance mechanism, particularly when bacteria overexpress efflux pumps and the concentration of antibiotic within the cell decreases to ineffective concentrations [1]. Moreover, efflux pumps are involved in the efflux of extracellular polymeric substances, which constitute bacterial biofilm, and quorum-sensing signals, which are responsible for virulence mechanisms [2].

Recently, our group has disclosed xanthones as inhibitors of bacterial efflux pumps, biofilm formation and quorum sensing [3], which has prompted us to investigate a series of xanthones associated with chiral moieties for their antimicrobial activity and efficacy in the reversal of antimicrobial resistance mechanisms. As such, a library of ten chiral derivatives of xanthones and six xanthone precursors were tested for these purposes. First, those compounds were experienced against four clinically relevant bacterial strains and three fungal strains (one yeast, one dermatophyte, and one filamentous fungi), with no compounds showing promising antibacterial or antifungal activity. The compounds were also tested for the synergy with antibiotics against antimicrobial-resistant bacteria, and one enantiomer and one precursor displayed synergy with a β-lactam in an extended-spectrum β-lactamase-producing strain of *Escherichia coli*.

Lastly, the compounds were tested for their potential of inhibiting bacterial efflux pumps in a Gram-positive and a Gram-negative strain with three different enantiomers showing activity. Further studies into the related mechanisms also showed that one enantiomeric pair presented very different results for biofilm formation inhibition but similar results in the inhibition of quorum-sensing.

Overall, the results obtained showed different results for different enantiomers, highlighting the important role of enantioselectivity in microbial resistance mechanisms.

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References

1. Durães, F.; Pinto, M.; Sousa, E. Medicinal chemistry updates on bacterial efflux pump modulators. *Curr. Med. Chem.* **2018**, *25*, 6030–6069, https://doi.org/10.2174/0929867325666180209142612.
2. Alav, I.; Sutton, J.M.; Rahman, K.M. Role of bacterial efflux pumps in biofilm formation. *J. Antimicrob. Chemother.* **2018**, *73*, 2003–2020. https://doi.org/10.1093/jac/dky042.
3. Durães, F.; Resende, D.I.S.P.; Palmeira, A.; Szemerédi, N.; Pinto, M.M.M.; Spengler, G.; Sousa, E. Xanthones Active against Multidrug Resistance and Virulence Mechanisms of Bacteria. *Antibiotics* **2021**, *10*, 600–616, https://doi.org/10.3390/antibiotics10050600.

7.31. Enantioresolution of Promethazine and Its Metabolites for Enantiomeric Profile in Metabolic Studies

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In recent decades, there has been an increase in the inappropriate use of pharmaceutical products at a global level. However, the risks are considered greater when these substances are chiral and marketed as racemate. The chirality of pharmaceutical products means that enantiomers may have different behaviors in terms of pharmacodynamics, pharmacokinetics and toxicity [1]. For these reasons, enantiomers and their metabolites must be treated as independent molecular entities, since one enantiomer may produce the desired therapeutic activity while the other may exhibit toxicity.

The use of antihistamines has been an example of the inappropriate rise of pharmaceuticals, as some are used in drugs of abuse. For example, the famous hallucinogenic drink "Purple Drank" that combines codeine and/or promethazine (PMZ) with soda has been winning new consumers around the world, mostly teenagers, being associated to serious health consequences and fatalities. PMZ is a chiral antihistaminic drug marketed as racemate that when used in high doses may cause severe toxicity effects [2].

The information about the enantioselectivity in toxicity of PMZ and its metabolites—namely, promethazine sulfoxide (PMZSO), desmonomethyl promethazine (DMPMZ), desmonomethyl promethazine sulfoxide (DMPMZSO) and the hydroxylated metabolite (PMZOH)—is scarce [2–4].

The aim of this work is to present a new enantioselective analytical method for monitoring the PMZ and their metabolites in in vitro metabolic studies. For that, the enantioseparation was evaluated in five different chiral analytical columns with amylose and cellulose carbamate as a chiral selector in normal and reversed elution mode. The amylose derivative showed good enantioselective and resolution for all target compounds within the same chromatographic conditions.

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Fund (ERDF), and the Project CHIRALSINTE-SE_APSFCT_IINFACTS_2021. Maria Miguel Coelho acknowledges her PhD grant provided by the FCT (SFRH/BD/146999/2019).

References

1. Coelho, M.M.; Fernandes, C.; Remião, F.; Tiritan, M.E. Enantioselectivity in Drug Pharmacokinetics and Toxicity: Pharmacological Relevance and Analytical Methods. *Molecules* **2021**, *26*, 3113, https://doi.org/10.3390/molecules26113113.
2. Peters, R.J.; Kelder, S.H.; Markham, C.M.; Yacoubian, G.S.J.; Peters, L.A.; Ellis, A. Drug testing in blood: Validated negative-ion chemical ionization gas chromatographic–mass spectrometric assay for determination of amphetamine and methamphetamine enantiomers and its application to toxicology cases. *J. Org. Chem.* **2003**, *44*, 1957–1960.
3. Gao, S.; Zhou, X.; Lang, L.; Liu, H.; Li, J.; Li, H.; Wei, S.; Wang, D.; Xu, Z.; Cai, H.; Zhao, Y.; Zou, W. Simultaneous determination of schisandrin and promethazine with its metabolite in rat plasma by HPLC-MS/MS and its application to a pharmacokinetic study. *Int. J. Anal. Chem.* **2019**, *2019*, 3497045.
4. Vanapalli, S.R.; Kambhampati, S.P.; Putcha, L.; Bourne, D.W. A liquid chromatographic method for the simultaneous determination of promethazine and three of its metabolites in plasma using electrochemical and UV detectors. *J. Chromatogr. Sci.* **2001**, *39*, 70–72.

7.32. Design and Synthesis of Azobenzene Photoswitches with Potential as VEGFR2 Inhibitors

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Angiogenesis is a tight controlled process in healthy adults but also plays a key role in tumor growth [1]. VEGFR2 is a dynamic and crucial tyrosine kinase receptor involved in angiogenesis [2]. Thus, targeting VEGFR2 with selective inhibitors can be regarded as a promising anticancer therapy and a useful strategy to understand the dynamic behavior of this enzyme. Photopharmacology is a powerful tool to reduce side effects in cancer therapy, since photoactive ligands are designed to interact with their targets only after light exposure [3,4].

In the present study, we aim to expand the toolbox of anti-angiogenic agents by developing new photoactivatable inhibitors based on known VEGFR2 inhibitors that can be exclusively activated in situ using light of biocompatible wavelength suitable for cells and, ultimately, for living tissues. These transformations will generate configurational isomers with distinct geometries displaying differentiated behavior when interacting with the target. For this purpose, a new sorafenib derivative (Figure 1), with an azobenzene photoswitch incorporated into the structure of the known VEGFR2 inhibitor, was synthesized and characterized for its photochemistry. The new compound exhibits the desired photoswitching properties for the pursued applications (biocompatible light excitation, high switching efficiency, high *E*-*Z*/*Z*-*E* conversion, good fatigue resistance, and thermal stability of *Z*-isomer).

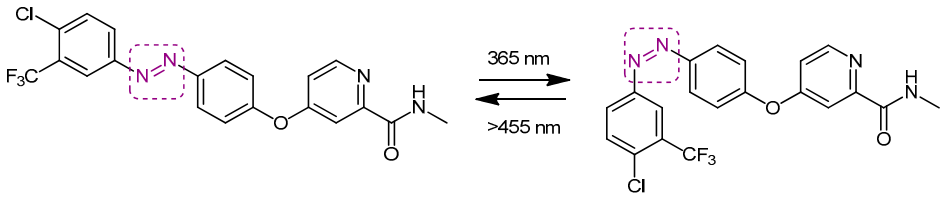

Figure 1. Photoswitchable sorafenib derivative.

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References

1. Eelen, G.; Treps, L.; Li, X.; Carmeliet, P. Basic and Therapeutic Aspects of Angiogenesis Updated. *Circ. Res.* **2020**, *127*, 310–329, https://doi.org/10.1161/CIRCRESAHA.120.316851.
2. Wang, X.; Bove, A.M.; Simone, G.; Ma, B. Molecular Bases of VEGFR-2-Mediated Physiological Function and Pathological Role. *Front. Cell Dev. Biol.* **2020**, *8*, 1314, https://doi.org/10.3389/fcell.2020.599281.
3. Fuchter, M.J. On the Promise of Photopharmacology Using Photoswitches: A Medicinal Chemist's Perspective. *J. Med. Chem.* **2020**, *63*, 11436–11447, https://doi.org/10.1021/acs.jmedchem.0c00629.
4. Lerch, M.M.; Hansen, M.J.; van Dam, G.M.; Szymanski, W.; Feringa, B.L. Emerging Targets in Photopharmacology. *Angew. Chemie Int. Ed.* **2016**, *55*, 10978–10999, https://doi.org/10.1002/anie.201601931.

7.33. New Facet on Porphyrins Cycloaddition Reactions and Heterodienes

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Cycloaddition reactions involving porphyrins have been extensively explored throughout the years, and the known versatility of the obtained adducts for different applications is responsible for the continuous interest on this type of strategy [1,2]. Porphyrins, when adequately substituted, can react either as 2π or 4π components in different cycloaddition approaches, namely in hetero Diels–Alder reactions [1,2]. Following our interest in this field and taking advantage of the well-known reactivity of phthalimidesulfenyl chloride to give access to α,α′-dioxothiones, from β-ketoesters [3–5], herein, we report our results concerning the reactivity of *meso*-tetraphenylporphyrins in the presence of this type of heterodienes (Figure 1). The synthetic conditions to obtain the required heterodiene precursor from phthalimidesulfenyl chloride with methylacetoacetate, the unexpected products obtained in its reaction with the selected porphyrin as well as mechanistic considerations will be presented and discussed.

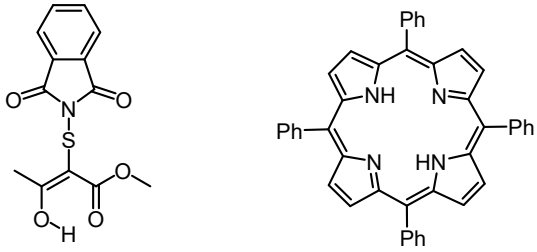

Figure 1. Representation of the structures of the heterodiene precursor (**left**) and a *meso*-tetraphenylporphyrin (**right**).

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References

1. Cavaleiro, J.A.S.; Neves, M.G.P.M.S; Tomé, A.C. Cycloaddition reactions of porphyrins. *Arkivoc* **2003**, *14*, 107–130, http://doi.org/10.3998/ark.5550190.0004.e11.
2. Tomé, A.C.; Neves, M.G.P.M.S.; Cavaleiro, J.A.S. Porphyrins and other pyrrolic macrocycles in cycloaddition reactions. *J. Porphyr. Phthalocyanines* **2009**, *13*, 408–414.
3. Capozzi, G.; Menichetti, S.; Nativi, C.; Rosi, A. Phthalimidesulfenyl chloride. Part 51. Reaction with enolizable carbonyl compounds and synthesis of functionalized thiones. *Tetrahedron* **1992**, *48*, 9023–9032.
4. Capozzi, G.; Franck, R.W.; Matioli, M.; Menichetti, S.; Nativi, C.; Valle, G. Phthalimidesulfenyl Chloride. 9. A Simple Access to α,α′-Dioxothiones, a New Class of Bis-heterodienes. Synthesis of 1,4-Oxathiin Systems *J. Org. Chem.* **1995**, *60*, 6416–6426.
5. Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C. Phthalimidesulfenyl Chloride.1 11. Generation, General Reactivity, and Synthetic Applications of o-Thioquinones. *J. Org. Chem.* **1997**, *62*, 2611–2615.

7.34. Carbon Dots from Tomato Industry Waste with Antibacterial Activity

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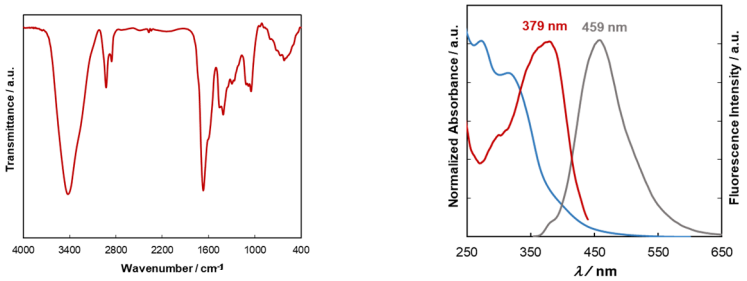
Tomato waste (TW) was directly employed as an eco-friendly source for the synthesis of fluorescent carbo dots (C-dots) using a sustainable hydrothermal carbonization method and ethylenediamine (ED) as a nitrogen additive. The antibacterial activity of TW carbon dots (TWCDs) revealed good and selective inhibitory capacity against E. coli in a concentration-dependent manner.

One of the most relevant manufacturing sectors in the Portuguese economy is the tomato processing industry [1], generating a large amount of tomato waste (e.g., tomato peels, seeds, and pulp; TW), which exhibit a high organic load (carbohydrates, lipids, etc.), representing a considerable pollution problem [2]. Carbon-containing fluorescent nanodots (CNDs) have recently appeared as candidates for several applications such as bioimaging,

catalysis and (bio)sensing due to their outstanding optical properties, low toxicity, high biocompatibility, and simple low-cost synthesis methods using a great diversity of green low-valued resources [3,4]. In addition, CNDs have been emerging as a new class of antimicrobial agents, showing potential antibacterial activity toward both Gram-negative and Gram-positive bacteria [5]. In this communication, we report the primary results concerning the sustainable synthesis of TWCDs using the one-pot hydrothermal carbonization (HTC) method. A preliminary evaluation of the antibacterial activity of TWCDs against *Escherichia coli* and *Staphylococcus aureus* is also presented.

After collection from a Portuguese tomato industry, TW was triturated, dried in an oven at 60 °C, and directly used to prepare fluorescent TWCDs by conventional HTC in a high-pressure reactor at 250 °C for 6 h using ED as additive with variable ED/TW mass ratio (0.08–0.32). After purification procedures (membrane filtration and extraction by organic solvents), TWCDs were isolated as aqueous brown dispersions and were characterized by FT-IR (Figure 1a) and ^{1}H NMR (not shown).



(**a**) (**b**)

Figure 1. FTIR (KBr) (**a**) and UV-Vis (blue), excitation (red, monitored at 460 nm) and emission (gray, λ_{exc} = 380 nm) spectra (**b**) of TWCDs.

The photophysical properties were studied by UV-Vis and fluorescence spectroscopy (Figure 1b). All compounds exhibited low to moderate quantum yields (Φ_F = 0.08–0.16, λ_{exc} = 380 nm) and great stability toward photobleaching.

The antimicrobial properties of TWCDs were evaluated against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria, using the disc-diffusion susceptibility method [6]. Three TWCDs batches prepared with different ED/TW mass ratios (0.08, 0.16 and 0.32) in several amounts (100, 500 and 1000 μg/disc) were tested (Figure 2). From the diameters of the inhibition zones obtained around each disc, after incubation of the cultures, it was found that TWCDs exhibited activity against *E. coli* that increased with C-dots mass (from 100 to 1000 μg/disc). The highest inhibitory capacity was observed for TWCDs prepared with an ED/TW mass ratio of 0.32, decreasing with ED reduction (from 0.16 to 0.08). No antibacterial activity against *S. aureus* was perceived for the TWCDs, not even for the highest concentration tested (Figure 2d).

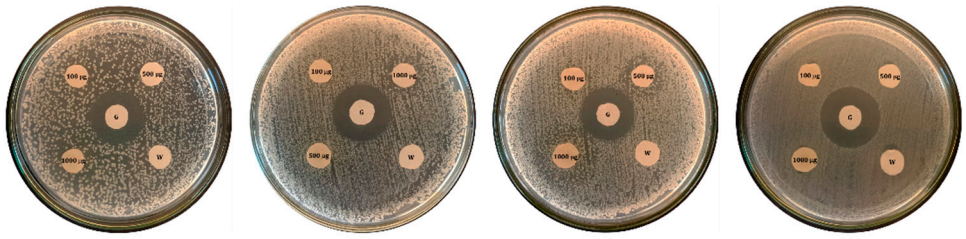


Figure 2. Antibacterial activity of TWCDs obtained with different ED/TW mass ratios (0.08 (**a**), 0.16 (**b**) and 0.32 (**c**,**d**)) in various concentrations (disc diffusion assay); gentamicin (G; 100 μg/disc) used as positive control and water (W) used as negative control.

TW was used successfully to produce fluorescent C-dots via a sustainable hydrothermal carbonization method. Preliminary results concerning antibacterial activity reveal that TWCDs can effectively and selectively inhibit the growth of *E. coli* in a concentration-dependent manner. Additionally, it has been observed that TWCDs inhibitory capacity depends on the ED/TW mass ratio used in its synthesis. Further studies concerning TWCDs synthesis and applications are now in progress.

Funding: We are grateful to FCT/MCTES for financial support (UIDB/00616/2022 and UIDP/00616/2022, and IPL (IPL/2021/NanoMate_ISEL). We thank Sugal Portugal for tomato waste supply.

References

1. Food and Agriculture Organization of the United Nations (FAO 2019). Available online: http://www.fao.org/home/en/ (accessed on 1 March 2022).
2. Vidyarthi, S.K.; Simmons, C.W. Characterization and management strategies for process discharge streams in California industrial tomato processing. *Sci. Total Environ.* **2020**, *723*, 137976.
3. Costa, A.I.; Barata, P.D.; Moraes, B.; Prata, J.V. Carbon Dots from Coffee Grounds: Synthesis, Characterization, and Detection of Noxious Nitroanilines. *Chemosensors* **2022**, *10*, 113.
4. Sousa, D.A.; Costa, A.I.; Alexandre, M.R.; Prata, J.V. How an Environmental Issue could Turn into Useful High-valued Products: The Olive Mill Wastewater Case. *Sci. Total Environ.* **2019**, *647*, 1097–1105.
5. Ghirardello, M.; Ramos-Soriano, J.; Galan, M.C. Carbon Dots as an Emergent Class of Antimicrobial Agents. *Nanomaterials* **2021**, *11*, 1877.
6. Jhonsi, M.A.; Ananth, D.A.; Nambirajan, G.; Sivasudha, T.; Yamini, R.; Bera, S.; Kathiravan, A. Antimicrobial activity, cytotoxicity and DNA binding studies of carbon dots. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2018**, *196*, 295–302.

7.35. Preliminary Studies with Small Molecules to Explore the Interaction of SARS-CoV-2 with Human Host Targets

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During viral recognition, viruses form a complex with receptors displayed at the surface of host cells, triggering subsequent steps necessary for cellular attachment, infection and viral replication [1]. Therefore, targeting viral recognition and attachment to host cellular receptors is a therapeutic strategy to develop affordable antivirals with broad-spectrum activity [2]. This is particularly relevant given the current viral epidemics and pandemics, such as the COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Angiotensin-converting enzyme 2 (ACE2) and cell-surface glucose-regulated protein 78 (GRP78) were identified as important host targets which facilitate the attachment and entry of different viruses into host cells. Several

virtual [3,4] and some in vitro studies [5–7] hypothesized that inhibiting the interaction between SARS-CoV-2 spike protein and cell surface (cs) ACE-2 and/or csGRP78 could possibly decrease the rate of viral infection. While csACE-2 is the key role receptor of SARS-CoV-2 [6], csGRP78 seems to be an essential host auxiliary factor. Indeed, other authors showed that the pretreatment of lung epithelial cells with a humanized monoclonal antibody against GRP78 reduced csACE-2 expression and SARS-CoV-2 spike viral entry and infection [7]. Thus, these two host proteins are suggested as putative molecular targets to fight SARS-CoV-2 infection.

In our group, several small molecules have been obtained in the last decade, and promising hits were discovered particularly with antiviral activity [8]. In this work, a docking study of an in-house library of about 300 bioactive compounds synthesized by Grupo de Produtos Naturais e Química Medicinal (CIIMAR/FFUP) was carried out on ACE-2 protein (PDB 6m17) and on structure binding domain (SBD) of GRP78 protein (PDB 5E84), using AutoDock Vina. Virtual screening revealed the interaction of 29 compounds with the ACE-2 and approximately 31 compounds with SBD GRP78 binding pockets, with better or equal docking scores than the positive control (ACE-2: 8.3 kcal/mol; GRP78: −8.4 kcal/mol). These promising compounds are mainly xanthones and steroids that present bulky, aminated or sugar hydroxylated moieties. Therefore, this new series of compounds deserves further exploration for its potential against SARS-CoV-2 infection.

Preliminary studies with two of the in silico hit compounds are being performed in cell lines to assess their effect on cytotoxicity (with the sulforhodamine B assay) and on the expression levels (by Western blot) of both cellular targets, ACE-2 and GRP78, with the long-term objective of verifying if these compounds may act as competitors of SARS-CoV-2.

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References

1. Aquino, R.S.; Park, P.W. Glycosaminoglycans and infection. *Front. Biosci.* **2016**, *21*, 1260–1277.
2. Mazzon, M.; Marsh, M. Targeting viral entry as a strategy for broad-spectrum antivirals. *F1000Res.* **2019**, *8*, F1000 Faculty Rev-1628, https://doi.org/10.12688/f1000research.19694.1.
3. Palmeira, A.; Sousa, E.; Köseler, A.; Sabirli, R.; Gören, T.; Türkçüer, İ.; Kurt, Ö.; Pinto, M.M.; Vasconcelos, M.H. Preliminary Virtual Screening Studies to Identify GRP78 Inhibitors Which May Interfere with SARS-CoV-2 Infection. *Pharmaceuticals* **2020**, *13*, 132.
4. Sternberg, A.; McKee, D.L.; Naujokat, C. Novel Drugs Targeting the SARS-CoV-2/COVID-19 Machinery. *Curr. Top. Med. Chem.* **2020**, *20*, 1423–1433.
5. Daniloski, Z.; Jordan T.X.; Wessels, H.H.; Hoagland, D.A.; Kasela, S.; Legut, M.; Maniatis, S.; Mimitou, E. P.; Lu, L.; Geller, E.; et al. Identification of Required Host Factors for SARS-CoV-2 Infection in Human Cells. *Cell* **2021**, *184*, 92–105.e16.
6. Wang, Q.; Zhang, Y.; Wu, L.; Niu, S.; Song, C.; Zhang, Z.; Lu, G.; Qiao, C.; Hu, Y.; Yuen, K.-Y.; et al. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* **2020**, *181*, 894–904.e9.
7. Carlos, A.J.; Ha, D.P. Yeh, D.-W.; Van Krieken, R.; Tseng, C.-C.; Zhang, P.; Gill, P.; Machida, K.; Lee, A. S. The chaperone GRP78 is a host auxiliary factor for SARS-CoV-2 and GRP78 depleting antibody blocks viral entry and infection. *J. Biol. Chem.* **2021**, *296*, 100759.
8. Lima, R.T.; Seca, H.; Palmeira, A.; Fernandes, M.X.; Castro, F.; Correia-Da-Silva, M.; Nascimento, M.S.J.; Sousa, M.E.; Pinto, M.; Vasconcelos, M.H. Sulfated Small Molecules Targeting EBV in Burkitt Lymphoma: FromIn SilicoScreening to the Evidence ofIn VitroEffect on Viral Episomal DNA. *Chem. Biol. Drug Des.* **2013**, *81*, 631–644, https://doi.org/10.1111/cbdd.12109.

7.36. Study of the Influence of Salicornia Ramosissima Ingestion on the Biochemical Profile of Shrimp (Penaeus vannamei)

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The Aquacombine project centers on cultivating and biorefining a type of salt-tolerant plant that can produce more food and plant material for bioenergy and biochemicals on marginal land [1]. Among these plants are species of the genus *Salicornia*. *Salicornia* species are halophytes that can grow on saline lands without freshwater for irrigation [2]. When grown as a vegetable, only the fresh tips are used, while the woody part of the plant is considered a residue. Thus, the need to value these residues and minimize their environmental impacts becomes evident through their use in different applications, such as health, food, and feed production for aquaculture. The present work focuses on this last topic and evaluates the effect of ingestion of *Salicornia ramosissima* at different percentages on the profile of secondary metabolites produced by aquaculture shrimp (*Penaeus vannamei*) using GC-MS and HPLC-MS [3]. In the preliminary studies carried out to analyze the polyphenolic constituents by HPLC-MS, no compounds were detected in the shrimp muscle. Our results, obtained by GC-MS, showed that the ingestion of *S. ramosissima* does not disturb the biochemical profile of shrimp (Figure 1), which suggests that its incorporation into the daily shrimp diet does do not affect its nutritional value.

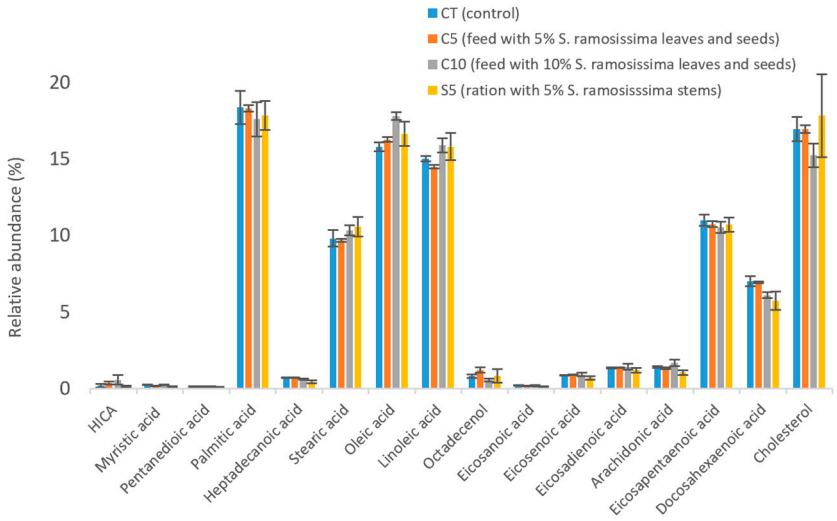

Figure 1. Secondary metabolites identified in shrimp.

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References

1. Thomsen, M.H. The AQUACOMBINE Project. Available online: https://www.aquacombine.eu/ (accessed on 10 November 2021).
2. Flowers, T.J.; Colmer, T.D. Salinity tolerance in halophytes*. *New Phytol.* **2008**, 945–963, https://doi.org/10.1111/j.1469-8137.2008.02531.x.
3. Oliveira, L.; Freire, C.S.R.; Silvestre, A.J.D.; Cordeiro, N.; Torres, I.C.; Evtuguin, D. Lipophilic extractives from different morphological parts of banana plant "Dwarf Cavendish." *Ind. Crops Prod.* **2006**, *23*, 201–211, https://doi.org/10.1016/J.INDCROP.2005.06.003.

7.37. SARS-CoV-2: Can (thio)Barbiturates Be a Potential Solution? An In Silico Study

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Since the beginning of the COVID-19 pandemic, SARS-CoV-2 virus has been responsible for the infection of more than 270 million people and more than 5.30 million deaths. Upon infection of hosts by the said virus, two different main types of viral entry are known: endocytosis or direct fusion between the virus and cell plasmatic membrane. In the first case, the SARS-CoV-2 spike protein needs another protein as its receptor, namely the angiotensin-converting enzyme 2 (ACE2) type 1. After entry, the viral genome is released in the cytoplasm and translated to viral polyproteins, which are processed by proteases, as papain-like protease (PLpro) or the main protease (Mpro), to form the replication complexes. These ways involve the targets that are considered the most encouraging for the development of antiviral drugs against SARS-CoV-2 infection and have been considered in in silico studies in this context. As there are still very few drugs approved by regulatory agencies for SARS-CoV-2 infection treatment and some just are effective for certain COVID-19 patients, it is clearly necessary to develop more efforts to discover new drugs or to adapt pre-existing ones. Recently, a computational study by molecular docking with auspicious results for some *N*,*N*-diethyl-2-thiobarbituric acid-based sulfonamides targeting the viral protein Mpro was published [1]. Bearing this in mind, we decided to evaluate the therapeutic potential for SARS-CoV-2 of several (thio)barbiturate derivatives, which were synthesized and described in previous works made by our research group [2–6], namely by their Mpro inhibition or ACE2 targeting. Molecular docking studies using GOLD software and analysis of absorption, distribution, metabolism, excretion, and toxicity parameters using webtools pkCSM and SwissADME were performed for eighty-two (thio)barbiturate derivatives. The obtained results pointed to a high potential of (thio)barbiturates with phenylhydrazinyl moiety (Figure 1) for targeting Mpro and ACE2 proteins.

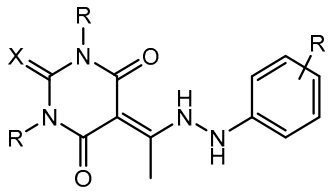

Figure 1. Most promising (thio)barbiturate backbone, from in silico studies, against Mpro and ACE2 proteins.

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References

1. Sarfraz, M.; Rauf, A.; Keller, P.; Qureshi, A.M. N,N′-dialkyl-2-thiobarbituric acid based sulfonamides as potential SARS-CoV-2 main protease inhibitors. *Can. J. Chem.* **2021**, *99*, 330–345.
2. Figueiredo, J.; Serrano, J.L.; Cavalheiro, E.; Keurulainen, L.; Yli-Kauhaluoma, J.; Moreira, V.M.; Ferreira, S.; Domingues, F.C.; Silvestre, S.; Almeida, P. Trisubstituted barbiturates and thiobarbiturates: Synthesis and biological evaluation as xanthine oxidase inhibitors, antioxidants, antibacterial and anti-proliferative agents, *Eur. J. Med. Chem.* **2018**, *143*, 829–842.
3. Figueiredo, J.; Serrano, J.L.; Soares, M.; Ferreira, S.; Domingues, F.C.; Silvestre, S.; Almeida, P. 5-Hydrazinylethylidenepyrimidines effective against multidrug-resistant Acinetobacter baumannii: Synthesis and in vitro biological evaluation of antibacterial, radical scavenging and cytotoxic activities, *Eur. J. Pharm. Sci.* **2019**, *137*, 104964.
4. Serrano, J.L.; Soeiro, P.F.; Reis, M.A.; Boto, R.E.F.; Silvestre, S.; Almeida, P. Synthesis and process optimization of symmetric and unsymmetric barbiturates C5-coupled with 2,1-benzisoxazoles, *Mol. Divers.* **2020**, *24*, 155–166.
5. Soeiro, P.F.; Serrano, J.L.; Paixão, J.A.; Boto, R.E.F.; Silvestre, S.; Almeida, P. The Synthesis of 2-Spiroindolin-3-one-(thio)barbiturates from 2,1-Benzisoxazoles: A Rearrangement Promoted by Thermal Conditions. *Synthesis* **2020**, *52*, 2065–2072.
6. Serrano, J.L.; Lopes, D.; Reis, M.J.A.; Boto, R.E.F.; Silvestre, S.; Almeida, P. Bis-thiobarbiturates as promising xanthine oxidase inhibitors: Synthesis and biological evaluation. *Biomedicines* **2021**, *9*, 1443.

7.38. Silver Nanoparticles Exert Harmful Effects in Human Monocytes

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Silver nanoparticles (AgNP) are the most widely produced and commercialized type of nanoparticles due to their unique antimicrobial and preservative properties. Nowadays, AgNP have gained access into our daily life, being applied in diverse sectors, from medicine to the food industry. Consequently, the potential human exposure and potential harmful effects in human health is a matter of increasing interest [1]. AgNP can enter the human body through many pathways, affecting the viability and activity of the "guard cells" of the immune system [2]. Monocytes are important components of the mononuclear phagocyte system, being characterized by their ability to identify possible foreign stimuli, via pattern recognition receptors. Monocytes can phagocytize, secrete chemokines, and proliferate in response to infection and injury [3]. Thus, it becomes imperative to evaluate the potential harmful effects of AgNP in these pivotal cells of the immune system. Therefore, the main objective of this work was to assess the cytotoxic and pro-inflammatory effects induced by AgNP (5, 10 and 50 nm) coated with two agents (polyvinylpyrrolidone (PVP) and citrate) in isolated human monocytes. For that purpose, human monocytes were isolated from human blood and then exposed to different concentrations ($\leq$ 25 μg/mL) of AgNP. Subsequently, the effects of PVP and citrate-coated AgNP (5, 10 and 50 nm) on cell viability, reactive species production, mitochondrial membrane potential and cytokines release were determined.

The results evidenced that the studied AgNP exert strong harmful effects in human monocytes through the induction of a powerful pro-inflammatory response that culminates in cell death. The observed effects were dependent on the AgNP concentration and on their size and coating, in which more pronounced cytotoxic effects with smaller PVP- coated AgNP (5 nm) were observed.

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References

1. Firdhouse, M.J.; Lalitha, P. Biosynthesis of Silver Nanoparticles and Its Applications. *J. Nanotechnol.* **2015**, *2015*, 829526, https://doi.org/10.1155/2015/829526.
2. Haase, H.; Fahmi, A.; Mahltig, B. Impact of silver nanoparticles and silver ions on innate immune cells. *J. Biomed. Nanotech.* **2014**, *10*, 1146–1156, https://doi.org/10.1166/jbn.2014.1784.
3. Chiu, S.; Bharat, A. Role of monocytes and macrophages in regulating immune response following lung transplantation. *Curr. Opin. Organ Transplant.* **2016**, *21*, 239–245, https://doi.org/10.1097/mot.0000000000000313.

7.39. Inhibition of Pancreatic Lipase and α-Amylase by Polyphenols in In Vitro Microanalysis Systems

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Obesity is a disease of epidemic proportions with an increasing trend. According to the latest data from the World Health Organization, in 2016, more than 1.9 billion people suffer from overweight, of which 650 million suffer from obesity. Statistical studies show that these numbers have tripled in the last 40 years [1]. Obesity is described as an abnormal or excessive state of adiposity accumulation due to excessive calorie intake [2]. Given the importance of lipids and carbohydrates from diet on the onset and development of obesity, their absorption offers a series of targets whose modulation can be explored for obesity treatment. Those include pancreatic lipase (PL) and α-amylase for their role in the absorption of lipids and carbohydrates, respectively. Pancreatic lipase is the enzyme responsible for the degradation of about 50 to 70% of dietary triglycerides [3], while α-amylase is the responsible for breaking down α-1,4 glycosidic bonds of starch [4]. Despite some inhibitors already being developed, namely orlistat as a pancreatic lipase inhibitor and acarbose as an α-amylase inhibitor, both are associated with low efficacy and undesirable side effects, including abdominal distension, flatulence, or oily stools. Thus, the search and development of new effective and safer agents able to control the caloric intake is of

great importance for obesity management and control. Polyphenols are naturally occurring and structurally diverse compounds and have diverse biological activities, such as anti-inflammatory, antioxidant, neuroprotective, antidiabetic and anti-obesity activities [5], which highlights their potential as lead compounds for the treatment of obesity. In the present study, a panel of structurally related polyphenols, including flavonoids, chalcones and 2-styrylchomones (2-SC), presenting hydroxyl (-OH), chloro (-Cl) and alkyl groups were chosen, and its inhibitory effects against the presented enzymes as well as its structure–activity relationship were evaluated. The results obtained for PL showed that the studied 2-SC achieved higher inhibitory activity when compared to the corresponding flavonoids and chalcones. Results also indicate that an extended alkyl group in the C-ring seems to be relevant to the inhibitory activity. Similarly, for α-amylase, 2-SC appears to be the group of polyphenols studied with the highest inhibitory effects, and the increase in the number of -OH substituents as well as the presence of a catechol group on the B-ring seems to confer greater inhibitory effects to the compounds. This work indicates that some of the tested polyphenols should be further explored as potential anti-obesity molecules.

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References

1. WHO. Obesity and Overweight. Available online: http://shorturl.at/ewET2 (accessed on June 2021).
2. National Institute of Diabetes; Digestive and Kidney Diseases. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. *Am. J. Clin. Nutr.* **1998**, *68*, 899–917, https://doi.org/10.1093/ajcn/68.4.899.
3. Birari, R.B.; Bhutani, K.K. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discov. Today* **2007**, *12*, 879–889, https://doi.org/10.1016/j.drudis.2007.07.024.
4. Proença, C.; Freitas, M.; Ribeiro, D.; Tomé, S.M.; Oliveira, E.F.T.; Viegas, M.F.; Araújo, A.N.; Ramos, M.J.; Silva, A.M.S.; Fernandes, P.A.; et al. Evaluation of a flavonoids library for inhibition of pancreatic α-amylase towards a structure-activity relationship. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 577–588, https://doi.org/10.1080/14756366.2018.1558221.
5. Li, A.-N.; Li, S.; Zhang, Y.-J.; Xu, X.-R.; Chen, Y.-M.; Li, H.-B. Resources and Biological Activities of Natural Polyphenols. *Nutrients* **2014**, *6*, 6020–6047, https://doi.org/10.3390/nu6126020.

7.40. Cosmeceutical Potential of the Green Macroalga Caulerpa Prolifera

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The present cosmeceutical industry has been gradually shifting its interest from products based on synthetic compounds to macroalgae-based products due to their interesting antiaging properties but also to their lower cytotoxicity and allergens content. Molecules

isolated from macroalgae already showed potential as either active cosmetic ingredients or key elements for the consistency of the cosmetic formulation [1]. In this regard, it is of foremost importance to keep studying the chemical composition of different algal species, aiming to find new compounds with cosmeceutical potential.

Caulerpa prolifera (Figure 1), a green macroalgae species which invaded the Azorean waters [2], is widely understudied in terms of phytochemical composition and cosmeceutical properties, so the present work aims to extract and determine the antiaging activities of *Caulerpa prolifera* components.

Figure 1. Underwater photo of *Caulerpa prolifera*.

The dry material was sequentially extracted by maceration with three solvents of increasing polarity (dichloromethane, acetone, and ethanol). The extracts obtained were then fractionated by solubility in different solvents, which was followed by fractionation with different chromatographic techniques, namely column chromatography (CC) and thin-layer chromatography (TLC). The extracts and fractions obtained were tested for their antioxidant and chelating activity and the inhibitory activity of elastase, collagenase, tyrosinase, and hyaluronidase.

The best result obtained was for the dichloromethane extract (CP1), which inhibited tyrosinase activity with an IC_{50} of 31.3 ± 0.37 µg/mL, which was followed by its ethyl acetate fraction (CP1.2) with an IC_{50} of 40.8 ± 0.21 µg/mL. In addition, fraction CP1.2.5 was active against elastase with an IC_{50} of 45.9 ± 0.75 µg/mL. Further experimental results and the respective discussion will be presented.

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References

1. Morais, T.; Cotas, J.; Pacheco, D.; Pereira, L. Seaweeds Compounds: An Ecosustainable Source of Cosmetic Ingredients? *Cosmetics* **2021**, *8*, 8, https://doi.org/10.3390/cosmetics8010008.
2. Cacabelos, E.; Faria, J.; Martins, G.M.; Mir, C.; Parente, M.I.; Gabriel, D.; Sánchez, R.; Altamirano, M.; Costa, A.C.; van Reine, W.P.; et al. First record of *Caulerpa prolifera* in the Azores (NE Atlantic). *Bot. Mar.* **2019**, *62*, 155–160, https://doi.org/10.1515/bot-2018-0075.

7.41. Machine Learning Methods to Predict the Terrestrial and Marine Origin of Natural Products

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In recent years, there has been a growing interest in studying the differences between the chemical and biological space represented by natural products (NPs) of terrestrial and marine origin [1]. NPs continue to be one of the most productive sources of chemical inspiration for the development of new drugs.

To learn more about these two chemical spaces, marine natural products (MNPs) and terrestrial natural products (TNPs), a machine learning (ML) approach was developed in the present work to predict three classes: MNPs, TNPs and a third class of NPs that appear in both the terrestrial and marine environments. In total, 22,398 NPs were retrieved from the Reaxys® database (Elsevier Information Systems GmbH, Frankfurt, Germany); from those, 10,790 molecules are recorded as MNPs, 10,857 as TNPs, and 761 NPs appear registered as both MNPs and TNPs [2]. Several ML algorithms, e.g., Random Forest, Support Vector Machines, and deep learning Multilayer Perceptron networks have been benchmarked. The best performance was achieved with a consensus classification model (CM), which predicted the external test set with an overall predictive accuracy up to 81%. The best model, CM, was also used for the virtual screening of the terrestrial and marine origin of NPs from the StreptomeDB 2.0 database [3] and the data set available in the work of Pye et al. [4], an extended compilation of 2877 NPs produced by the genus Streptomyces and 5486 microbial and marine-derived NPs, respectively. The results suggest that the implemented ML models could be used with success to predict the terrestrial and marine origin of NPs, and in this way, we could understand the chemical space defined by MNPs, TNPs or both, but also in virtual screening to define the applicability domain of QSAR models of MNPs and TNPs, as was completed in a virtual screening of the StreptomeDB 2.0 database and the Pye data set (Figure 1) [2].

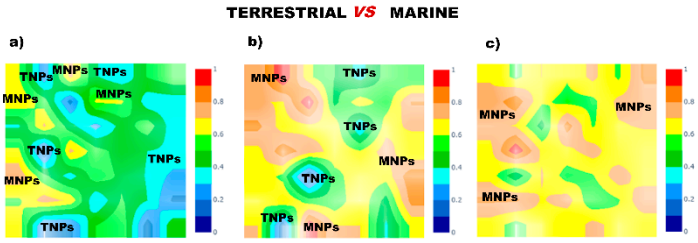

Figure 1. Generative Topographic Mapping (GTM) terrestrial and marine origin of NPs landscape for: (**a**) the test set; (**b**) the StreptomeDB 2.0 database; and (**c**) the Pye data set.

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References

1. Shang, J.; Hu, B.; Wang, J.; Zhu, F.; Kang, Y.; Li, D.; Sun, H.; Kong, D.-X.; Hou, T. Cheminformatic Insight into the Differences between Terrestrial and Marine Originated Natural Products. *J. Chem. Inf. Model.* **2018**, *58*, 1182–1193.
2. Pereira, F. Machine Learning Methods to Predict the Terrestrial and Marine Origin of Natural Products. *Mol. Inf.* **2021**, *40*, 2060043.
3. Klementz, D.; Doering, K.; Lucas, X.; Telukunta, K. K.; Erxleben, A.; Deubel, D.; Erber, A.; Santillana, I.; Thomas, O. S.; Bechthold, A.; Guenther, S. StreptomeDB 2.0—an extended resource of natural products produced by streptomycetes. *Nucleic Acids Res.* **2016**, *44*, D509–D514.
4. Pye, C.R.; Bertin, M.J.; Lokey, R.S.; Gerwick, W.H.; Linington, R.G. Retrospective Analysis of Natural Products Provides Insights for Future Discovery Trends. *Proc. Natl. Acad. Sci.* **2017**, *114*, 5601–5606.

7.42. Evaluation of Cytotoxic and Pro-Inflammatory Effects of Silver Nanoparticles in Gastrointestinal Tract In Vitro Models: Potential Protective Effect of Flavonoids

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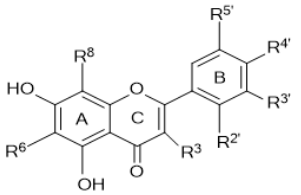
The growing developments in the nanoscience and nanotechnology fields have resulted in several consumer products, many of which are routinely used in our daily life. Among the 1814 products listed in the Nanotechnology Consumer Products Inventory, 438 (24%) contain silver nanoparticles (AgNP) [1].

Due to their unique antimicrobial properties, AgNP were incorporated in all phases of food production process (processing, packaging and storage). Therefore, the human dietary intake of AgNP may result in an extensive oral exposure leading to unpredicted harmful effects in the gastrointestinal tract (GIT) [2], which should be considered in the risk assessment and management of these materials. In the present study, the toxic effects of polyethyleneimine (PEI)-coated AgNP (4 and 19 nm) were evaluated in GIT-relevant cells (Caco-2 cell line as a model of human intestinal cells, and human neutrophils as a model of the intestinal inflammatory response). Moreover, regarding the putative cytotoxic and pro-inflammatory effects of AgNP, and considering that flavonoids represent the most common group of plant polyphenols, with recognized antioxidant and anti-inflammatory effects [3], this study also evaluated the putative protective action of some dietary flavonoids (Figure 1) against such harmful effects.



1) $R^{2'}$, $R^{5'}$, R^3, R^6, R^8=H; $R^{4'}$=OH; $R^{3'}$=OMe (Chrysoeriol)
2) $R^{2'}$, $R^{5'}$, R^3, R^6, R^8=H; $R^{3'}$=OH; $R^{4'}$=OMe (Diosmetin)
3) $R^{2'}$, $R^{5'}$, R^3, R^6, R^8=H; $R^{3'}$, $R^{4'}$=OH (Luteolin)
4) $R^{2'}$, $R^{5'}$, R^6, R^8=H; R^3, $R^{4'}$, $R^{3'}$=OH (Quercetin)
5) $R^{2'}$, R^6, R^8=H; $R^{3'}$, $R^{4'}$, $R^{5'}$, R^3=OH (Myricetin)
6) $R^{3'}$, $R^{5'}$, R^6, R^8 =H; $R^{2'}$, $R^{4'}$, R^3 =OH (Morin)
7) $R^{2'}$, $R^{5'}$, R^8 =H; $R^{3'}$, $R^{4'}$, R^3, R^6 =OH (Quercetagetin)
8) $R^{2'}$, $R^{5'}$, R^6 =H; $R^{3'}$, $R^{4'}$, R^3, R^8 = OH (Gossypetin)

Figure 1. Chemical structures of the studied flavonoids.

The obtained results showed that AgNP of 4 and 19 nm effectively induced Caco-2 cell death by apoptosis with the concomitant production of nitric oxide, irrespective of the size. It was also observed that AgNP induced a human neutrophil oxidative burst. Interestingly, some flavonoids, namely quercetin and quercetagetin, prevented the deleterious effects of AgNP in both cell types. Overall, the data of the present study provide a first insight into the promising protective role of flavonoids against the potentially toxic effects of AgNP at the intestinal level [4].

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References

1. Vance, M.E.; Kuiken, T.; Vejerano, E.P.; McGinnis, S.P.; Hochella, Jr. M.F.; Rejeski, D.; Hull, M.S. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein J. Nanotechnol.* **2015**, *6*, 1769–1780. https://doi.org/10.3762/bjnano.6.181.
2. Bouwmeester, H.; Dekkers, S.; Noordam, M.Y.; Hagens, W.I.; Bulder, A.S.; de Heer, C.; ten Voorde, S.E.; Wijnhoven, S.W.; Marvin, H.J.; Sips, A.J. Review of health safety aspects of nanotechnologies in food production. *Regul. Toxicol. Pharmacol.* **2009**, *53*, 52–62. Https://doi.org/10.1016/j.yrtph.2008.10.008
3. Ribeiro, D.; Freitas, M.; Lima, J.L.; Fernandes, E. Proinflammatory Pathways: The Modulation by Flavonoids. *Med. Res. Rev.* **2015**, *35*, 877–936. https://doi.org/10.1002/med.21347.
4. Rufino, A.T.; Ramalho, A.; Sousa, A.; Oliveira, J.M.P.; Freitas, P.; Gomez, M.A.G.; Pineiro-Redondo, Y.; Rivas, J.; Carvalho, F.; Fernandes, E.; Freitas, M. Protective Role of Flavonoids against Intestinal Pro- Inflammatory Effects of Silver Nanoparticles. *Molecules* **2021**, *26*, 6610. https://doi.org/10.3390/molecules26216610.

7.43. Computer-Assisted Design of Indoloisoquinolines as Potential DNAG4–Helicase Interaction Inhibitors

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Guanine-rich DNA or RNA sequences may form a noncanonical higher-order structure called G-quadruplexes (G4). The structural features of G4 have been described to promote genomic instability in DNA replication, modulate transcription and translation and have been found with high prevalence in promoter regions of many cancer-related genes such as c-MYC [1,2]. G4s are transient structures that can be unfolded by helicases, which is a protein family that binds and remodels nucleic acid structures and nucleic acid protein complexes. Some helicases, such as DHX36, have a preference for binding and unwinding G4 nucleic acid structures [3]. In previous reports, G4 structure stabilization by small organic molecules has shown promising results as an anticancer drug target [2,4]. However, many difficulties related to the lipophilicity and lack of specificity toward specific G4s have been found. To overcome these obstacles, in this project, we propose to design, synthesize and evaluate indoloisoquinoline derivatives as potential inhibitors of the interaction between c-MYC:G4 and its negative regulator [5]. The indoloisoquinoline core was combined with a library of purchasable fragments to create a final database of compound derivatives. This dataset was then used in a computational Molecular Docking screening campaign, using the c-MYC:G4 structure in complex with DHX36 [5], to identify the most promising c-MYC:G4-helicase interaction inhibitors, which will afterwards be prioritized for synthesis. The synthesized compounds will then be evaluated, using in vitro assays, for binding and selectivity to the c-MYC-G4. The obtained results will be integrated in additional structure:function evaluations and guide new computational predictions, synthesis and functional validation.

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References

1. Nakanishi, C.; Seimiyaab, H. G-quadruplex in cancer biology and drug discovery. *Biochem. Biophys. Res. Commun.* **2020**, *8*, 45–50.
2. Paulo, A.; Castillo, C.C.; Neidle, S. Targeting Promoter Quadruplex Nucleic Acids for Cancer Therapy. In *Comprehensive Medicinal Chemistry III*, 3rd ed.; Chackalamannil, S., Rotella, D., Ward, S.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 308–340.
3. Balaratnam, S.; Schneekloth Jr., J.S.; Transcriptional regulation of MYC through G-quadruplex structures. In *Annual Reports in Medicinal Chemistry*; Academic Press: Cambridge, MA, USA, 2020; Volume 54, pp. 361–407.
4. Cadoni, E.; Magalhães, P.R.; Emídio, R.M.; Mendes, E.; Vítor, J.; Carvalho, J.; Cruz, C.; Victor, B.L.; Paulo, A. New (Iso)quinolinyl-pyridine-2,6-dicarboxamide G-Quadruplex Stabilizers. A Structure-Activity Relationship Study. *Pharmaceuticals* **2021**, *14*, 669, https://doi.org/10.3390/ph14070669
5. Chen, M.C.; Tippana, R.; Demeshkina, N.A.; Murat, P.; Balasubramanian, S.; Myong, S.; Ferré-D'Amaré, A.R. Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36. *Nature* **2018**, *558*, 465–483.

7.44. The Synthesis of 2-Substituted Quinoline and Pyrrolo[1,2-a]quinolinium Salt from Tetrahydroquinoline by Pyrrolidone Cleavage

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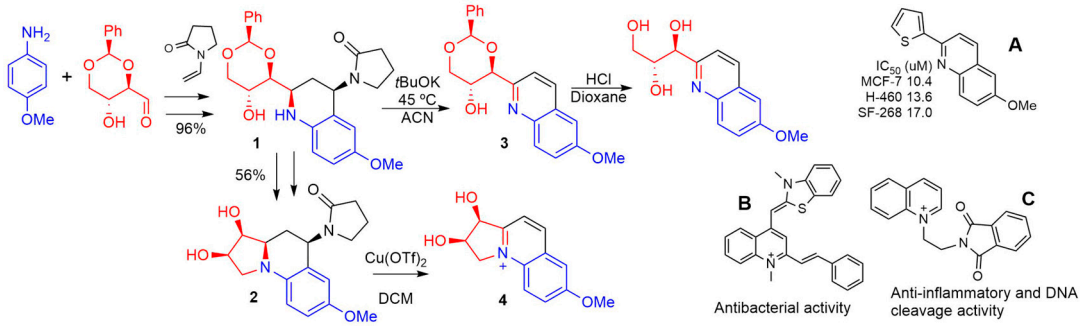
Quinolines are present in a large number of plants and fungi that humanity has used for many centuries in folk medicine. The quinoline ring plays an important role in the search for new anticancer drugs as their derivatives. Quinolines have shown excellent biological results through different mechanisms of action such as cell growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation [1]. The 2-substituted quinoline (**A**) demonstrates good anticancer activity in MCF-7 (mammary glands), H-460 (non-small cell lung), and SF-268 (CNS) cancer cell lines, and low cytotoxicity in a non-cancer cell line [2]. Quinolinium salts also show good properties, e.g., (**B**) as an antibiotic with antibacterial activity similar to vancomycin and methicillin, and they display low cellular toxicity [3]. Compound **C** demonstrates anti-inflammatory activity and DNA cleavage activity [4].

In this work, we synthesized a 2-substituted quinoline (**3**) and a pyrrolo[1,2-*a*]quinolinium salt (**4**) from tetrahydroquinoline (THQ) **1** and hexahydropyrroloquinoline (HHPQ) **2**, whose syntheses were reported previously [5]. The 2-substituted quinoline 3 was obtained from **1** by treatment with potassium *t*-butoxide in acetonitrile at 45 °C. The quinolinium salt **4** was obtained from **2** by smooth oxidative cleavage of the pyrrolidone group with copper triflate in dichloromethane in 82% yield for the last step (Scheme 1).



Scheme 1. Synthesis of 2-substituted quinoline and pyrrolo[1,2-*a*]quinolinium salt.

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References

1. Jain, S.; Chandra, V.; Jain, P.K.; Pathak, K.; Pathak, D.; Vaidya, A. Comprehensive review on current developments of quinoline-based anticancer agents. *Arab. J. Chem.* **2019**, *8*, 4920–4946. https://doi.org/10.1016/j.arabjc.2016.10.009.
2. Kouznetsov, V.V.; Ruiz, F.A.R.; Mendez, L.Y.V.; Gupta, M.P. Simple C-2-Substituted Quinolines and their Anticancer Activity. *Lett. Drug Des. Discov.* **2012**, *9*, 680–686. https://doi.org/10.2174/157018012801319544.
3. Sun, N.; Du R.-L.; Zheng, Y.-Y.; Guo, Q.; Cai, S.-Y.; Liu, Z.-H.; Fang, Z.-Y.; Yuan, W.-C.; Liu, T.; Li, X.-M.; Lu, Y.-J.; Wong, K.-Y. Antibacterial activity of 3-methylbenzo[d]thiazol-methylquinolinium derivatives and study of their action mechanism. *J. Enzyme Inhib. Med. Chem.* **2018**, *33*, 879–889.
4. Singh, P.; Kumar, R.; Singh, A.K.; Yadav, P.; Khanna, R.S.; Vinayak, M.; Tewari, A.K. Synthesis and crystal structure of quinolinium salt: Assignment on nonsteroidal anti-inflammatory activity and DNA cleavage activity. *J. Mol. Struct.* **2018**, *1163*, 262–269.
5. Drogalin, A.; Fortes, A.G.; Alves, M.J. Synthesis of functionalized tetrahydroquinolines, and its further transformation into hexahydropyrroloquinoline-2,3-diol by aminocyclization. Presentesd at XXIV Encontro Luso-Galego de Quimíca, Porto, Portugal, 21–23 November 2018. https://doi.org/10.13140/RG.2.2.12336.81921.

7.45. Application of Eugenol in Medicinal Organic Synthesis: Contribution to the Creation of Molecular Diversity and New Bioactive Substances

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The molecular modification of bioactive and abundant natural products is a strategy that is frequently used in Medicinal Chemistry projects aimed at discovering new drug candidates. Our research group has focused its studies on the generation of new analogues and derivatives of easily accessible phenylpropanoids such as eugenol. The main objective

of our work is to contribute to the discovery of new active agents with antimicrobial, antiparasitic or antitumor properties that can be used for medicinal or agrochemical applications. In this context, starting from eugenol, dihydroeugenol and isoeugenol, we obtained groups of bioactive compounds with different structural patterns, especially focusing on heterocycles. Using classical organic reactions, we explored substitution, oxidation and reduction reactions that resulted in functionalization and coupling products such as *N*-Mannich bases, sulfonamides, triazoles, coumarins, benzoxazoles, benzisoxazolinones, dihydrobenzofurans, and glycosides, amongst others (Figure 1). Biological studies with these new compounds highlighted some important candidates as new antifungal agents against opportunistic species of *Candida* spp. [1–3], resistant Gram-negative bacteria and rapid growing mycobacteria [4,5], MCF7 cancer cells [6] and protozoa such as *Leishmania* sp. and *Trypanosoma cruzi* [7–9], which cause serious neglected tropical diseases.

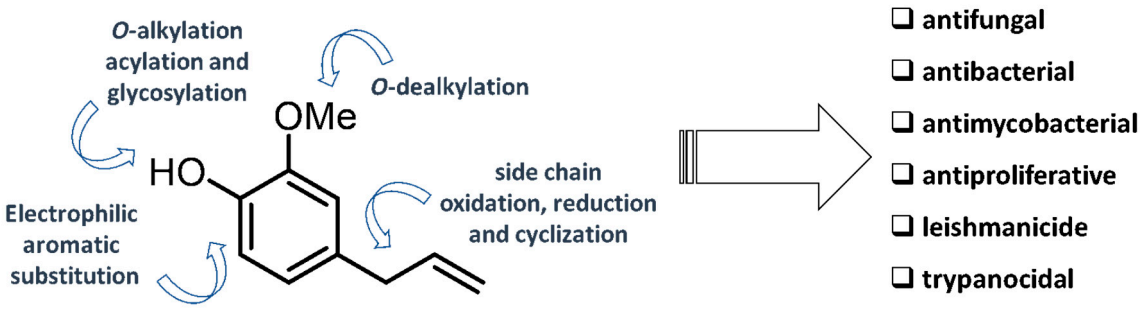

Figure 1. Structural changes in eugenol leading to new bioactive compounds.

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References

1. Fregnan, A.M.; Júnior, A.A.F.; Alvarenga, D.J.; Oliver, J.C.; Silva, N.C.; Coelho, L.F.; Dias, A.L.T.; Dias, D.F.; de Souza, T.B.; Carvalho, D.T. A New 1,2-Benzisoxazolin-3-one Synthetized from Eugenol Shows Anti- Candida Spp. Activity, Specially Against Opportunistic Candida Glabrata. *Curr. Bioact. Compd.* **2021**, *17*, 1–9, https://doi.org/10.2174/1573407216999200723114241.
2. Abrão, P.H.O.; Pizi, R.B.; De Souza, T.B.; Silva, N.C.; Fregnan, A.M.; Silva, F.N.; Coelho, L.F.L.; Malaquias, L.C.C.; Dias, A.L.T.; Dias, D.; et al. Synthesis and Biological Evaluation of New Eugenol Mannich Bases as Promising Antifungal Agents. *Chem. Biol. Drug Des.* **2014**, *86*, 459–465, https://doi.org/10.1111/cbdd.12504.
3. de Souza, T.B.; Brito, K.M.D.O.; Silva, N.C.; Rocha, R.P.; de Sousa, G.F.; Duarte, L.P.; Coelho, L.F.L.; Dias, A.L.T.; Veloso, M.P.; Carvalho, D.T.; et al. New Eugenol Glucoside-based Derivative Shows Fungistatic and Fungicidal Activity against Opportunistic*Candida glabrata*. *Chem. Biol. Drug Des.* **2015**, *87*, 83–90, https://doi.org/10.1111/cbdd.12625.
4. dos Santos, T.; Coelho, C.; Elias, T.; Siqueira, F.; Nora, E.; De Campos, M.; De Souza, G.; Coelho, L.; Carvalho, D. Synthesis and Biological Evaluation of New Eugenol-Derived 1,2,3-Triazoles as Antimycobacterial Agents. *J. Braz. Chem. Soc.* **2019**, https://doi.org/10.21577/0103-5053.20190038.
5. de Souza, T.B.; Raimundo, P.O.B.; Andrade, S.F.; Hipólito, T.M.M.; Silva, N.C.; Dias, A.L.T.; Ikegaki, M.; Rocha, R.P.; Coelho, L.F.L.; Veloso, M.P.; et al. Synthesis and antimicrobial activity of 6-triazolo-6-deoxy eugenol glucosides. *Carbohydr. Res.* **2015**, *410*, 1–8, https://doi.org/10.1016/j.carres.2015.04.002.
6. Azevedo-Barbosa, H.; Ferreira-Silva, G..; Silva, C.F.; de Souza, T.B.; Dias, D.F.; de Paula, A.C.C.; Ionta, M.; Carvalho, D.T. Phenylpropanoid-based sulfonamide promotes cyclin D1 and cyclin E down-regulation and induces cell cycle arrest at G1/S transition

in estrogen positive MCF-7 cell line. *Toxicol. Vitr.* **2019**, *59*, 150–160, https://doi.org/10.1016/j.tiv.2019.04.023.
7. Brancaglion, G.A.; Toyota, A.E.; Machado, J.V.C.; Júnior, A.F.; Silveira, A.T.; Boas, D.F.V.; dos Santos, E.G.; Caldas, I.S.; Carvalho, D.T. In vitro and in vivo trypanocidal activities of 8-methoxy-3-(4-nitrobenzoyl)-6-propyl-2*H* -cromen-2-one, a new synthetic coumarin of low cytotoxicity against mammalian cells. *Chem. Biol. Drug Des.* **2018**, *92*, 1888–1898, https://doi.org/10.1111/cbdd.13362.
8. Pelozo, M.F.; Lima, G.F.S.; Cordeiro, C.F.; Silva, L.S.; Caldas, I.S.; Carvalho, D.T.; Lavorato, S.N.; Hawkes, J.A.; Franco, L.L. Synthesis of New Hybrid Derivatives from Metronidazole and Eugenol Analogues as Trypanocidal Agents. *J. Pharm. Pharm. Sci.* **2021**, *24*, 421–434, https://doi.org/10.18433/jpps31839.

7.46. Potential of Acetophenone-1,2,3-Triazole Hybrids in Modulation of Marine Biofouling

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Marine biofouling is a natural process caused by the attachment of micro and macroorganisms that occurs on marine vessels and other submerged structures, which leads to several economic issues for maritime industries, as well as environmental and health concerns. Although some booster biocides have been used in biofouling control, they were found to have toxicity on marine non-target organisms [1]. Therefore, it is urgent to develop new eco-friendly alternatives.

Acetophenones have been described as modulators of several biological activities, including antifouling (AF) activity [2]. Moreover, the 1,2,3-triazole ring has shown to display antifouling and anticorrosive properties [3]. Therefore, in this work, acetophenones were hybridized with the 1,2,3-triazole ring and other chemical substrates through the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC).

The obtained compounds were firstly screened against the settlement of a representative macrofouling mussel *Mytilus galloprovincialis* and on biofilm-forming marine bacteria. The most promising compounds were further evaluated for their ability to inhibit the growth of *Navicula* sp. microalgae. Three compounds showed significant inhibition of the settlement of mussels' larvae. Three other compounds were able to inhibit the growth of *Roseobacter litoralis* biofilm-forming bacteria. Interestingly, one acetophenone hybrid was found to display complementary AF activity against macrofouling mussel larvae and microalgae *Navicula* sp. The ecotoxicity assay against marine non-target organism *Artemia salina* revealed that the most potent compounds were less toxic than the biocide Econea®, being considered potential eco-friendly AF agents.

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References

1. Wang, K.-L.; Wu, Z.-H.; Wang, Y.; Wang, C.-Y.; Xu, Y. Mini-Review: Antifouling Natural Products from Marine Microorganisms and Their Synthetic Analogs. *Mar. Drugs* **2017**, *15*, 266.
2. Jung, S.; Sidharthan, M.; Lee, J.; Lee, H.; Jeon, J.; Park, T.; Yoon, J.; Jeon, J.; Shin, H. Antifouling efficacy of a controlled depletion paint formulation with acetophenone. *Sci. Mar.* **2017**, *81*, 449–456.
3. Kantheti, S.; Narayan, R.; Raju, K.V.S.N. The impact of 1,2,3-triazoles in the design of functional coatings. *RSC Adv.* **2015**, *5*, 3687–3708.

7.47. Targeted Photodynamic Therapy: New Photosensitizers That "Click"

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Photodynamic therapy (PDT) is a technique that by combining light, a photosensitizer (PS), and oxygen, produces reactive oxygen species (ROS) that destroy nearby cells [1,2]. This classifies PDT as a light-delivered treatment, which gives it several advantages over more traditional anticancer therapies, as it is minimally invasive, has low mutagenic potential and low systemic toxicity [3,4]. This latter is caused by the light-delivery of PDT because, while tumor selectivity is one of the sought after characteristics of an ideal PS, most do not have a tumor/normal tissue ratio high enough to completely eliminate the tissue photosensitivity and damage to healthy tissue surrounding tumors [1]. To overcome these selectivity problems, targeting strategies have been used involving either passive methods (PS modification; delivery vehicles; serum proteins association) or active targeting (conjugation to endogenous ligands, antibodies, or growth factors) [2].

Herein, we present a set of novel chlorins that were synthesized through (8π+2π) cycloaddition [5] and have shown phototoxic activity against the MDA-MB-231 human adenocarcinoma cell line. When testing the chlorins against this highly aggressive, triple-negative breast cancer model, the results revealed activity in the nanomolar range, with the most promising chlorin having an IC50 < 100 nM. Furthermore, the second most promising chlorin is a "clickable" derivative of the latter (IC50 ≈100 nM), which was developed to be easily conjugated through "click" chemistry to facilitate the synthesis of selective PS for targeting.

In addition to presenting these novel PS, we also propose the synthesis of a folate-PS conjugate as proof-of-concept, which is expected to increase the PS selectivity. While several targeted-PDT strategies have been developed and produced positive results, a targeted-PS has yet to reach the clinic [2,6,7], which is why it is important to continue developing conjugating strategies that can be applied to other targets and/or other chlorins known to present a strong activity—in our case through β-functionalization effectively achieved with ($8\pi + 2\pi$) cycloaddition, which can later undergo "click" chemistry [5].

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References

1. Dąbrowski, J.M.; Arnaut, L.G. Photodynamic Therapy (PDT) of Cancer: From Local to Systemic Treatment. *Photochem. Photobiol. Sci.* **2015**, *14*, 1765–1780, https://doi.org/10.1039/C5PP00132C.
2. Hudson, R.; Boyle, R.W. Strategies for Selective Delivery of Photodynamic Sensitisers to Biological Targets. *J. Porphyr. Phthalocyanines* **2004**, *8*, 954–975, https://doi.org/10.1142/S1088424604000325.
3. Milla Sanabria, L.; Rodríguez, M.E.; Cogno, I.S.; Rumie Vittar, N.B.; Pansa, M.F.; Lamberti, M.J.; Rivarola, V.A. Direct and Indirect Photodynamic Therapy Effects on the Cellular and Molecular Components of the Tumor Microenvironment. *Biochim. et Biophys. Acta* **2013**, *1835*, 36–45, https://doi.org/10.1016/j.bbcan.2012.10.001.
4. Gierlich, P.; Mata, A.I.; Donohoe, C.; Brito, R.M.M.; Senge, M.O.; Gomes-da-Silva, L.C. Ligand-Targeted Delivery of Photosensitizers for Cancer Treatment. *Molecules* **2020**, *25*, 5317, https://doi.org/10.3390/molecules25225317.
5. Pereira, N.A.M.; Fonseca, S.M.; Serra, A.C.; Pinho e Melo, T.M.V.D.; Burrows, H.D. [8π+2π] Cycloaddition of Meso-Tetra- and 5,15-Diarylporphyrins: Synthesis and Photophysical Characterization of Stable Chlorins and Bacteriochlorins. *European J. Org. Chem.* **2011**, *2011*, 3970–3979, https://doi.org/10.1002/ejoc.201100465.
6. Chen, S.; Zhao, X.; Chen, J.; Chen, J.; Kuznetsova, L.; Wong, S.S.; Ojima, I. Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated Endocytosis and Drug Release. *Bioconjug. Chem.* **2010**, *21*, 979–987, https://doi.org/10.1021/bc9005656.
7. Akhtar, M.J.; Ahamed, M.; Alhadlaq, H.A.; Alrokayan, S.A.; Kumar, S. Targeted Anticancer Therapy: Overexpressed Receptors and Nanotechnology. *Clin. Chim. Acta* **2014**, *436*, 78–92, https://doi.org/10.1016/j.cca.2014.05.004.

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